Understanding the impact of intensity modulated radiation therapy on dental and oral tissues in oropharyngeal cancer patients

Patel, Vinod

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Understanding the impact of intensity modulated radiation therapy on dental and oral tissues in oropharyngeal cancer patients.

Vinod Patel

BDS (Hons), MFDS RCS Ed, MOralSurg RCS Eng

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II Swami Shreeji II

Unidentified flying objects, abominable snowmen, the Loch Ness monster and human cancer viruses

-Medical World News, 1974,

on four "mysteries" widely reported and publicised but never seen

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Abbreviations

3D-CRT – Three-dimensional conformal radiotherapy

ADC – Analogue to Digital Convertor

ADHS - Adult Dental Health Survey

BOT – Base Of Tongue

CAA – Customised Angiogenesis Analyzer

DMFT – Decayed Missing Filled Teeth

DPT – Dental Panoramic Tomograph

EBT – External Beam Therapy

ES – Ex-Smoker

FOV – Field Of View

GSTFT – Guys & St Thomas' NHS Foundation Trust

Gy - Grays

HN – Head and Neck

HNC – Head and Neck Cancer

HBL – Horizontal Bone Loss

HBOT – Hyperbaric Oxygen Therapy

HPV – Human Papilloma Virus

IMRT – Intensity Modulated Radiotherapy Treatment

IMPT – Intensity Modulated Proton Therapy

LC – Laryngeal Cancer

MDM - Multi-Disciplinary Meeting

MRONJ – Medication Related Osteonecrosis of the Jaw

MVD – Microvasculature Density

NPC – Nasopharyngeal Cancer

NS – Non-Smoker

OCC – Oral Cavity Cancer

OCT – Optical Coherence Tomography

ONJ - Osteonecrosis of the jaw

OPC – Oropharyngeal Cancer

ORN – Osteoradionecrosis

PBRT – Proton Beam Radiation Therapy

PENTOCLO – Pentoxifylline-Tocopherol-Clodronate

PTX – Pentoxifylline

PVe – Pentoxifylline & Vitamin E/Tocopherol

QoL – Quality of life
RIF – Radiation Induced Fibrosis
RT – Radiotherapy
RTOVI – Real Time Optical Vascular Imaging
SCC – Squamous Cell Carcinoma
TILs – Tumour Infiltrating Lymphocytes
TNM7 – Tumour Node Metastasis Version 7
TNM8 - Tumour Node Metastasis Version 8
Ve – Vitamin E/Tocopherol

Dental Notation

1 – central incisor
2 – lateral incisor
3 - canine
4 – 1st premolar
5 – 2nd premolar
6 – 1st molar
7 – 2nd molar
8 – 3rd molar
UR – upper right
URQ – upper right quadrant
UL – upper left
ULQ – upper left quadrant
LR – lower right
LRQ – lower right quadrant
LL – lower left
LLQ – lower left quadrant

Abstract

Osteoradionecrosis (ORN) of the jaw remains one of the most feared late toxicity complications of head and neck radiotherapy (RT). With the introduction of intensity modulated radiation therapy (IMRT) many hypothesised the complications would be consigned to the history books based on the ability to deliver more focused and targeted radiation. However, in contrast, the opposite has been seen with ORN still persisting and arguably increasing. The latter has been identified particularly in the oropharyngeal cancer (OPC) group more than any other sub-site. In the IMRT era, incidence of ORN in head and neck cancer (HNC) was approximately 5.5%. However, when considering the OPC alone it is almost double (10.5%). The two main factors for ORN; the dentition and RT dose are both of clinical significance in explaining the rise of ORN in OPC patients.

The overall dental status of OPC patients is vastly superior compared to many of the other common HNC patients. OPC patient often present with more teeth with less active dental disease compared to other HNC sub-sites. This fact is further strengthened when the OPC patients are divided by their HPV status with HPV positive patients having a vastly superior dentition. This is based on them presenting with more teeth including third molars, more complex restored teeth and less periodontal disease. Hence, though they have suffered dental disease burden, efforts have been made to retain their teeth. This trend is opposite to the various other common HNC sub-sites who present with fewer teeth and active dental disease in line with a pattern of dental neglect and low oral health priority. Hence, the retention of teeth leaves a significant risk factor for the development of ORN.

RT to the oropharynx via IMRT inadvertently includes the jaws, particularly in the HPV positive patient who presents with late stage disease due to its indolent nature. Hence large areas of the jaws are subjected to doses in excess of 30 Grays (Gy) following which radiation induced microvasculature depletion is evident in the hard and soft tissue of the oral cavity. This phenomenon worsens with increasing RT doses and over time. Both apply to the OPC patient. The posterior dentition often receives in excess of 40 Gy which is the minimum threshold for ORN. In addition, favourable survival particularly in the HPV positive group allows the progressive development of radiation induced fibrosis. Hence, high tooth retention, irradiated dento-alveolar bone with limited reparative potential and fibrosed oral mucosa as a poor protector creates a finely balanced oral conflict for the development of ORN with increase time.

It is therefore evident that the OPC patient is vulnerable to developing ORN possessing all the key factors for creating a 'perfect storm'. With HPV positive patients being a clear outlier to the typical HNC patient and the epidemic rise of this tumour it only compounds the complication further in a group already presenting with an elevated risk.

Hypothesis

OPC patients are at an elevated risk of developing radiation induced fibrotic changes such as ORN

Null hypothesis

Oropharyngeal cancer patients are not at an elevated risk of developing radiation induced fibrotic changes such as ORN

Objectives of the studies

The objectives were

1. To establish the pre-RT dental status of HNC patients.
2. To determine whether the pre-RT dental status of OPC aligns with other HNC sub-sites.
3. To determine whether the pre-RT dental status of OPC differ based on oncological demographics.
4. To identify dental dosimetry in OPC patients based on IMRT
5. To evaluate the impact of RT doses to the microvasculature of hard and soft tissue in OPC patients.

Specific outcomes

The overall objective was to translate the study data to provide:

1. Evidence of differing dental status of OPC patients compared to other HNC sub-sites.
2. Reasoning and support towards the clinical scenario of increasing ORN rates in the OPC patients.
3. Reporting the microvasculature changes of the soft and hard tissues in response to RT doses.

Chapter 1

Introduction A: Oropharyngeal cancer and intensity modulated radiation treatment

1.1 Defining the oropharynx

The pharynx commonly known as the throat is sub-divided into three sections. Commencing at the most superior region is the nasopharynx. Inferior to this is the oropharynx, which is directly posterior to the oral cavity. Below the oropharynx is the hypopharynx.

The anatomical boundaries of the oropharynx incorporate the base of tongue, the inferior surface of the soft palate and uvula, the anterior and posterior tonsillar pillars, the glossotonsillar sulci, the pharyngeal tonsils, and the lateral and posterior pharyngeal walls (Greene et al., 2002).

1.2 Oropharyngeal cancer

Oropharyngeal cancer (OPC) is a sub-division of head and neck cancer (HNC) which is the sixth most common malignancy worldwide (Vigneswaran & Williams, 2014). Squamous cell carcinoma (SCC) comprises approximately 90% of all HNCs (Ahmedin Jemal et al., 2011) however for OPC this rises to 95% (CRUK, 2018).

OPC is classified using the international cancer staging system known as TNM (Tumour, Nodes, Metastases). The most recent version; TNM8 has seen significant changes in how OPC patients are staged based on whether the tumour is positive or negative for the presence of human papilloma virus (HPV). However, this proposed re-staging has not been fully adopted as switching will lead to a change in tumour management, which has not yet been validated via robust evidence. Hence, in the UK, TNM7 staging continues to be the gold standard. For the purpose of future transition, it has become routine practice to collect both TNM7 and TNM8 classification data simultaneously to assist in building evidence.

1.2.1 Risk factors

Traditional risk factors for HNC, particularly for oral cavity cancer (OCC) and OPC of SCC origin are tobacco and alcohol use (Hashibe et al., 2007; Hashibe et al., 2009). In the UK, smoking rates have declined from 46% (1974) to 20% (2010) (NHS, 2015; ONS, 2011) while in contrast, per capita alcohol consumption has increased from 3.9 L/year (1950)

to 9.4 L/year (2004) (BMA, 2008). This appears consistent with the increasing incidence of cancers at sites with greater exposure to alcohol (e.g oral cavity) but smaller increases at sites more strongly associated with tobacco smoking (e.g. larynx) (Hashibe et al., 2007; Hashibe et al., 2009).

Beyond these traditional factors, HPV has increasingly been recognized as a risk factor. The oncogenic potential of HPV was first described in cervical cancer in the 1980s (Dürst et al., 1983). However, in the last three decades, it has been identified as a risk factor for HNC specifically in OPC and to a much lesser extent OCC and laryngeal cancer. In such cases they are often referred to as HPV positive OPC. This sub-category of patients is distinctly different to the OPC HPV negative patients whose risk factors continue to be driven by the traditional factors of alcohol and smoking (Hashibe et al., 2009).

1.2.2 Human papilloma virus positive oropharyngeal cancer

Despite relatively successful public health strides regarding tobacco and excessive alcohol use, incidence of OPC has continued to rise (Chaturvedi et al., 2013). The increase has been seen principally in HPV positive OPC patients, many of whom do not smoke and consume alcohol within the recommended limits (Chaturvedi et al., 2013; Drinkaware, 2019). Hence, the impact of HPV has been recognised as central to this continual rise in incidence of OPC. Trends toward sexual practices (such as increasing numbers of lifetime vaginal or oral sex partners, casual sex participation, no barrier use during vaginal or oral sex, and history of a sexually transmitted disease) (Chaturvedi et al., 2013; D'Souza et al., 2009; D'Souza et al., 2007; Gillison et al., 2008) have been proposed as possible explanations.

Approximately 200 HPV types exist based on viral genome sequence (de Villiers, 2013). Of these 16 (HPV 11, 16, 18, 31, 32, 33, 44, 45, 51, 52, 53, 56, 57, 58, 59, 81) have been isolated from HN tumours (Anantharaman et al., 2013; Kreimer et al., 2005; Michaud et al., 2014; Steinau et al., 2014; Viens et al., 2016). The oropharynx, sub-site however has proven to show a strong relationship to HPV and particularly HPV16 accounting for 87-90% of all primary causes (Kreimer et al., 2005; C. Ndiaye et al., 2014). HPV18 and HPV33 are essentially responsible for the remaining cases (C. Ndiaye et al., 2014). The switch from infection to tumour is protracted highlighting the indolent nature of the virus. In a Scandinavian case-control study involving almost 900,000 HPV16 positive individuals,

the presence of the virus (sero-positivity) was observed on average of 9.4 years prior to the onset of disease and was associated with a 14-fold increased risk of OPC (Mork et al., 2001). Others have reported even longer latency periods ranging from 12 to 30 years (Guo et al., 2017; Martín-Hernán et al., 2013). Furthermore, oropharyngeal HPV infection can occur in the absence of epithelial abrasion (Perry, 1994) which is in contra-distinction to other sites of HPV infection. This distinct characteristic has led to HPV positive OPC cases surpassing the incidence of HPV-positive cervical cancer (Chaturvedi et al., 2011; Panwar et al., 2014) making it the most frequently diagnosed cancer caused by HPV (CDC, 2019). Due to the limited vaccine uptake and extended latency period of HPV it is estimated that this trend will continue until 2060 (Gillison et al., 2015).

Hence, HPV positive OPC, is recognized as a distinct neoplastic entity with a unique molecular, histopathological, epidemiological, and clinical profile (CGARN, 2012; Fakhry et al., 2008; Gillison et al., 2008) compared to HPV negative OPC which has the same traits as other traditional HN SCCs.

1.3 Prevalence and incidence of OPC

A meta-analysis assessing the presence of HPV in a healthy population found an overall prevalence of 7.7% for all types of HPV with 1.4% being high-risk HPV16 (Tam et al., 2018). Assessment by gender identified a much higher rate in men with the prevalence being 9.3% compared to 5.5% in women (Kreimer et al., 2010). Country development status also influenced prevalence with developed countries having a 7.3% prevalence while in developing countries this was 3.6% (Kreimer et al., 2010). Even with oral HPV infection having a lower prevalence than cervicogenital HPV infection in healthy individuals it continues to be an important concern given its oncogenicity and the rising incidence of OPC (Kreimer et al., 2010).

Though OPC and OCC are separate sub-sites for HNC they have often been discussed jointly likely due to numerous commonalities such as risk factors and site proximity. Cancer at these sites has a global footprint with an estimated 400,000 incident cases and 223,000 deaths during 2008 (Ferlay et al., 2010). However, the incidence of OCC has been declining in most part of the world consistent with smoking cessation while OPC is increasing with a divergent pattern due to HPV infection. This illustrates the distinction

between them (Chaturvedi et al., 2013). On the current trend, Chaturvedi et al estimated that by 2020 the incidence of HPV-positive OPC will be greater than the incidence of cervical cancer and by 2030 half of all HNCs will be related to HPV (Chaturvedi et al., 2011).

1.3.1 Worldwide

An estimated 85,000 cases of OPC occurred worldwide in 2008, and at least 22,000 of these were HPV positive (de Martel et al., 2012). Though the number may seem small compared to other cancers it is the trend that has led to the call that HPV positive OPC is a new epidemic (Marur et al., 2010). Evidence is that between 1998-2004, HPV positive OPC increased 225% in North America while simultaneously HPV negative OPC decreased by 50% (Chaturvedi et al., 2011). It is unclear whether this increasing incidence is a global phenomenon or whether it is restricted to certain countries (Chaturvedi et al., 2013). It is well documented in North America, Europe and Australia (Chaturvedi et al., 2011; A. M. Hong et al., 2010) but not well established in South America, Africa, and Asia (Chaturvedi et al., 2008). For the latter 3 continents population-based studies are limited but also there are different cultural sexual practices and continued high tobacco consumption which may also obscure these trends (Chaturvedi et al., 2008).

In countries where OPC has been reported to be increasing it is estimated to be rising 5% annually (Blomberg et al., 2011; Chaturvedi et al., 2011). In contrast, OCC has been declining in high-income or developed nations (Chaturvedi et al., 2013). Over the past 20 years both these trends has been identified in Australia (A. M. Hong et al., 2010), Canada (Auluck et al., 2010), Denmark (Blomberg et al., 2011), Finland (Blomberg et al., 2011; Chaturvedi et al., 2011), the Netherlands (Braakhuis et al., 2009), Norway (Jon Mork et al., 2010), Sweden (Hammarstedt et al., 2006), the United States (Chaturvedi et al., 2008), and the United Kingdom (Reddy et al., 2010). This HPV infection phenomenon had led to estimations that it accounted for 38-72% (de Martel et al., 2012; H. Mehanna et al., 2013) of OPC in these countries compared with 13-17% of OPCs in less developed economies of the world (de Martel et al., 2012).

1.3.2 National

In England and Wales there are approximately 9,200 HNC each year of which 90% are SCCs (DAHNO, 2014). DAHNO (Data for Head and Neck Oncology) aims to report basic information in association with HNC. In their latest report which assessed the year 2014, 2439 OPC were registered accounting for almost 30% of all HNCs and second to OCC by a mere 245 cases (DAHNO, 2014).

The UK has seen a doubling in incidence of OPC between 1990 and 2006 which has doubled again between 2006 and 2010 (Mehanna. et al., 2016). While the UK incidence has increased by over 100% between 2002-2011 the incidence of laryngeal cancer has increased only by 9.3% (Schache et al., 2016). Hence, OPC incidence was projected to increase in England by 239% from 2011 to 2025, and if correct OPC would compose 35% of all HNCs at this time point (Louie et al., 2015).

In England OPC almost tripled in men and doubled in women in the period between 1995 and 2011 (ONS, 2019). Simultaneously, in women a significant decline in lung cancer incidence was reported (Chaturvedi et al., 2013) highlighting the rise of OPC impacted by HPV and not by smoking which has been falling. The proportion of HPV-positive cases was higher in men than in women (54.3% vs.44.4%) and decreased with increasing age (< 44y = 69.2%: > 75y = 37.2%) (Schache et al., 2016). The mean age of patients with HPV-positive disease was 57.4 years compared to 61.4 years for HPV-negative cases (Schache et al., 2016) indicating that the condition affects a younger population.

1.3.3 Regional & local

Though the rise of OPC has been identified at all population levels the total disease burden varies considerably by geographical region (Mehanna et al., 2016; Näsman et al., 2009; Ndiaye et al., 2014; Rietbergen et al., 2013; Stein et al., 2014). This is evident in the study by Schache et al (Schache et al., 2016) which showed a range (35.4 – 67.5%) of HPV OPC prevalence in the UK for various major cities.

The increase has been experienced within our HNC service at Guys & St Thomas' NHS Foundation Trust (GSTFT) (Figure 1.1). The hospital provides a tertiary care service and is a major cancer service provider for south east London. It remains important to

acknowledge that these trends exist within the GSTFT patient cohort as the subsequent research specifically focuses on this population within the Trust. Interestingly, dividing the south east London sector further by boroughs shows varying proportions of HPV positive and negative OPCs (Figure 1.2).

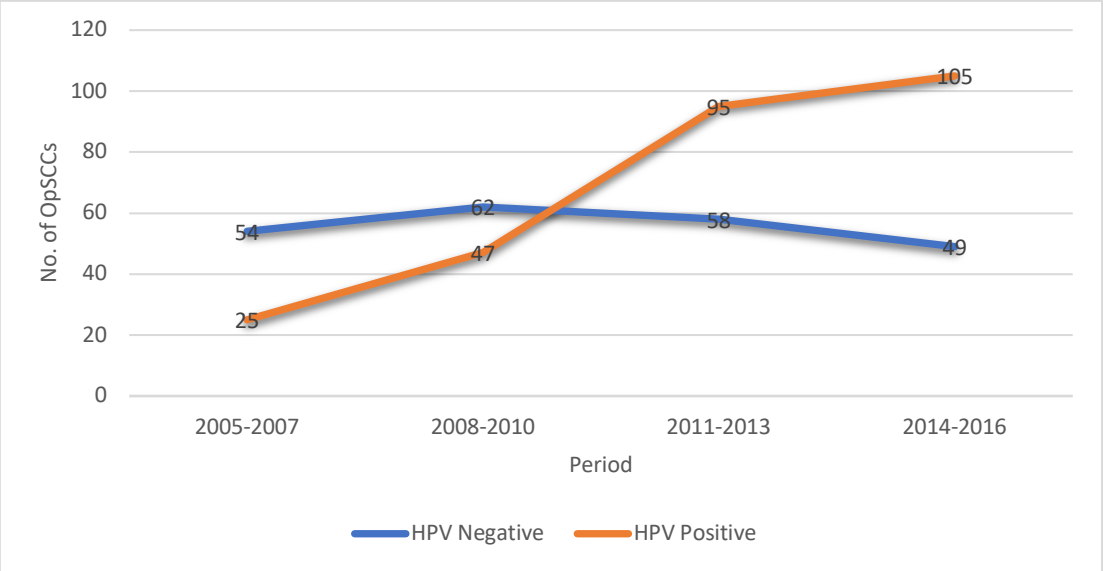


Figure 1.1 Number of biopsy confirmed HPV positive and HPV negative OPC presenting to GSTFT from 2005 to 2016. It highlights the rising trend of HPV positive OPC with simultaneous decline of HPV negative OPC (S Thavaraj, 2019).

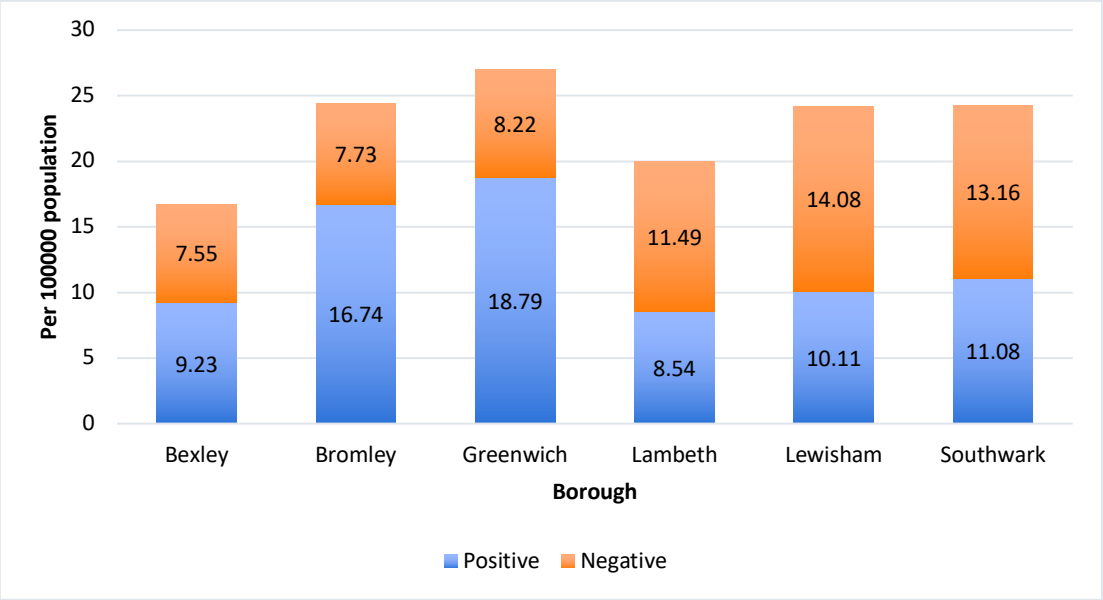


Figure 1.2: Prevalence of HPV positive and HPV negative OPC via boroughs in south east London presenting to GSTFT (S Thavaraj, 2019).

1.3.4 Oropharyngeal cancer patient profile

For HPV negative OPC the traditional profile of high tobacco use (Hong et al., 2013) and excessive alcohol intake persists. In contrast, HPV positive OPC patient profile is now typically middle aged (40-59) (Chaturvedi et al., 2013; Chaturvedi et al., 2011) white, male (Chaturvedi et al., 2013), non-smoker (Hong et al., 2013), reporting little to no oropharyngeal symptoms and presenting with a cystic level II neck lump. The latter is often considered to be a hallmark of HPV-related SCC, usually with sub-clinical primaries in the oropharynx especially the base of tongue (Strojan et al., 2013). The consistent trend that characterises HPV positive OPC is a young healthy population (age < 60 years) with no traditional adverse life style habits (Chaturvedi et al., 2013; Chaturvedi et al., 2008; Chaturvedi et al., 2011).

1.4 Radiotherapy

Radiotherapy (RT) works on the principle of passing ionising radiation through tissues and inducing damage to the DNA, so preventing further proliferation or death of the tumour cells. It is a well-established treatment modality used across a wide spectrum of cancers.

Though the intention is for RT to target tumour cells it is inevitable that healthy tissue will also be irradiated and therefore the treatment is non-discriminatory. In order to amplify the proportion of healthy cells over tumour cells, the total dose is divided and delivered into smaller doses called fractions. This approach allows healthy cells to repair the damage caused and proliferate in the gaps between radiation thus giving them a better chance of survival (Baskar et al., 2012).

In HNC, RT is routinely used either as a primary treatment or as an adjuvant measure following surgery. In the curative setting, RT is the treatment of choice for pharyngeal cancer with the addition of chemotherapy in larger tumours. Its use in surgery is often following tumour resection in large tumours or where clearance margins are minimal or not successfully achieved.

1.4.1 Evolvement of radiotherapy delivery

Historically, two-dimensional (2D) RT was used and planned based on plain radiography for tumour location. 2D RT consisted of a single beam from between one to four directions and plans were relatively simple.

Advances of this technique led to the introduction of three-dimensional (3D), or CT-based, planning (Tsien, 1955). Modern RT has evolved from non-site-specific techniques using bony anatomy and hand-drawn blocking towards specialized planning incorporating three-dimensional reconstructions of images and computer optimization algorithms (Bucci et al., 2005). This advancement accounted for axial anatomy and complex tissue contours such as the hourglass shape of the neck and shoulders (Bucci et al., 2005).The ability to delineate normal organs has meant a concerted effort can be made to avoid damage to healthy and non-targeted tissue (Bucci et al., 2005). Though 3D conformal RT (3DCRT) allowed better RT delivery to irregular shapes it involved a set of fixed beams often of uniform intensity. This itself was a significant limitation particularly for complex areas such as the HN region.

Advancement of 3DCRT has led to intensity-modulated radiation (IMRT) which is now routinely used worldwide.

1.4.2 IMRT

IMRT like 3DCRT also requires the use of a CT scan to map the tumour area and any surrounding key structures. This technique allows the operator to control the number of fields and intensity of each RT dose being delivered to the tissues within that field (IMRTCWG, 2001). IMRT can be either fixed beam or volume modulated arc therapy that can sculpt RT. Treatment planning is optimised with the assistance of a high-speed computer software (inverse planning or automated optimization), which intelligently selects the most appropriate beam directions and shapes (IMRTCWG, 2001). By modulating both the number of fields and the intensity of RT within each field there is limitless possibilities to sculpt RT dose (Figure 1.3) relevant to biologic principles such as altered fractionation, chemosensitization, and molecular targeting (IMRTCWG, 2001). An advantage is that it provides the opportunity to spare important non-target structures from the harmful effects of radiation (Wu et al., 2000; Xia et al., 2000). The caveat to this however, is that since more fields of radiation are used then a larger area of tissue is exposed to radiation (Bucci et al., 2005).

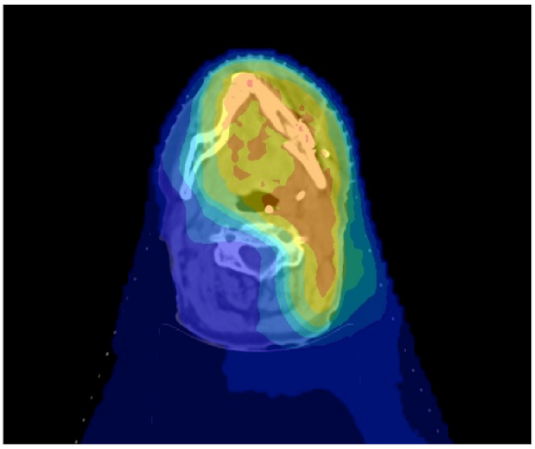


Figure 1.3: Shows a typical IMRT axial view scan with associated colour dosimetry

1.4.2.1 IMRT in head and neck cancer

IMRT has been proposed as an ideal delivery system for HNC to target tumours within the complex geometry of this region and limit the severity of radiation-associated toxicity. In many instances, the distance between the tumour and critical structures such as optic apparatus, inner ear, or salivary gland is no more than a few millimetres (IMRTCWG, 2001). RT to these HN regions lead to undesirable acute and late toxicities and are

recognized in the oncology sector as the worst seen in the field (IMRTCWG, 2001). Due to the potential to decrease the dose to normal tissue and therefore spare toxicity, IMRT has been utilized in the HNC area since its inception (IMRTCWG, 2001). The PARSPORT (Nutting et al., 2011) study examined sparing the parotid glands via IMRT and significantly reduces the incidence of xerostomia with improved quality of life (QoL). This result has consistently been shown in other trials (Chao et al., 2004; A. Eisbruch et al., 1999; N. Lee et al., 2002). Studies also report a reduction in severe early and late toxicities when IMRT was used, such as acute dysphagia and mucositis, weight loss, trismus, neck fibrosis and hearing loss (Gupta et al., 2012; Gupta et al., 2011; Peng et al., 2012). However, despite the enthusiasm and selective advantages attributed to IMRT in HNC it became apparent there was "beam path" toxicity to numerous non-targeted areas which were unobserved in previous RT eras (Rosenthal et al., 2008).

A recent systematic review and meta-analysis (Gupta et al., 2018) concluded the quality of evidence regarding the superiority of IMRT over conventional techniques for disease-related endpoints is rather low due to relative lack of power and inconsistency of results precluding robust conclusions. But a number of smaller studies have reported apparent improvements of IMRT over 2D-RT/3D-CRT (Graff et al., 2007; Vergeer et al., 2009) which have been recognised and embraced leading to IMRT being the delivery system of choice.

1.4.2.2 Radiotherapy and oropharyngeal cancer

OPC has notably become less of a surgical disease as cure rates with organ preservation therapy are widely recognized to equal those achieved with surgery followed by post-operative RT (Calais et al., 1999; Denis et al., 2004).

There are no good head-to-head comparisons of primary surgery and chemo-RT particularly in advance OPC. Currently, the treatment of HPV positive and HPV negative OPC is exactly the same as there is no evidence at present that patients with HPV positive and HPV negative OPC should be treated differently. With the rising HPV positive OPC incidence and excellent prognosis, in the low risk group there is a concerted effort to address the best practice in managing this tumour group and whether de-escalation of treatment is feasible. A multi-centre randomised study; PATHOS (UKCRN ID 18645)

(ClinicalTrials.gov, 2019) is currently investigating the optimum management of HPV positive OPC. In contrast, CompARE (ISRCTN41478539) (ISRCTN, 2020) is investigating whether escalating treatment will result in better outcomes in HPV negative and high risk HPV positive OPC who currently have a poorer prognosis.

Guidance on management of OPC has been outlined in the 5th edition of the UK Multi-Disciplinary Guidelines for HNC (BAHNO, 2016). Early stage (T1–T2 N0 M0) OPC is ideally treated by single modality therapy, either primary surgery or RT (Mehanna. et al., 2016). When RT is utilised a total dose equivalent of 70 Gy in 35 fractions is used in radical treatment. When the schedule is hypofractionated, typically 65–66 Gy in 30 fractions is frequently used (Mehanna. et al., 2016). Prophylactic RT should be given to the ipsilateral cervical lymph nodes for lateralised (e.g. tonsillar) tumours and to both sides of the neck for non-lateralised tumours (Mehanna. et al., 2016).

In advanced OPC (T3–T4 N0 and T1–T4 N1–N3) there has been a shift towards primary RT with additional chemotherapy as part of an 'organ preservation' strategy (Mehanna. et al., 2016). A RT dose equivalent of 70 Gy in 2 Gy fractions with concurrent cisplatin chemotherapy is considered standard for stage III and IV OPC. RT to levels Ib–IVa, V(a,b) and the retropharyngeal nodes (level VIIa) at the level of the oropharynx is generally recommended in a lymph node positive neck (Mehanna. et al., 2016). Controversy exist regarding whether the contralateral neck should be treated in patients with lateralised OPC with advanced nodal disease (N2+) which is dependent on local practice (Mehanna. et al., 2016). The debate is one of interest considering patients with HPV related OPC are a demographically growing portion of domestic cases and often present with level II lymphadenopathy (MDAndersonHNCSWG, 2017).

Surgery can be considered for advanced tumours but may not be deliverable due tumour size or extent. The indications for post-operative chemo-RT depends on pathological risk factors for recurrence and is reserved for patients with extra-capsular invasion or microscopically involved (<1 mm) surgical resection margins around the primary tumour or both (Mehanna. et al., 2016).

1.4.2.3 Chemotherapy and oropharyngeal cancer

Chemotherapy alone is not a treatment modality used in HNC but rather an adjunct to RT for local control, organ preservation with continued organ function and to decrease the incidence of sub-clinical micro-metastatic spread. Its use in OPC is commonly in advanced stages (Mehanna. et al., 2016).

In a large meta-analysis (MACH-NC) assessing in excess of 17,000 patients, concomitant chemotherapy (given during RT) was shown to improve locoregional control rates and was associated with a 6.5 per cent increase in survival ($p<0.0001$) (Blanchard et al., 2011; Pignon et al., 2009). A Cochrane review regarding the use of chemotherapy in OPC found evidence of statistically significant benefit in the addition of concomitant chemotherapy to post-operative RT (Furness et al., 2010).

1.5 Survival

Much of the controversy in the management of OPC is based on a clear and consistent overall survival outcome evidently based on HPV status. Despite presentation with advanced nodal disease, survival is improved in patients with HPV positive OPC compared with patients with HPV negative OPC (Ang et al., 2010; Ang & Sturgis, 2012; Li et al., 2003; O'Sullivan et al., 2012). HPV positive OPC have a 58% reduction in the risk of death compared with HPV-negative OPC. HPV positive patients also have a 3-year overall survival rate of 82.4% compared with 57.1% ($p<0.001$) for HPV negative disease (Ang et al., 2010). Within our unit patients with HPV positive OPC and a reassuring PET-CT had an estimated 3-year progression-free survival rate of 91.7% (85.2%-98.2%), compared to 66.2% (41.5%-90.9%) for patients with HPV negative tumours (Bird et al., 2016). Survival times within GSTFT for OPC (Figure 1.4 & 1.5) are comparable to those published in the literature across the UK and globally. Hence, a clear distinction can be identified and is well recognised between survival for HPV positive and HPV negative patients.

In addition, recent evidence highlighted that for patients treated for HPV positive or HPV negative OPC, the time to treatment initiation affects outcome, with the strongest effect on overall survival impacting HPV negative patients (Grønhøj et al., 2018). Hence, OPC are often placed on an expedited pathway where treatment in commenced within 14 days of diagnosis to achieve the best prognosis.

	Mean				Median			
			95% CI				95% CI	
	Estimate	**SE**	**Lower**	**Upper**	**Estimate**	**SE**	**Lower**	**Upper**
HPV Negative (months)	40.2	3.5	33.4	47.0	22.0	2.6	16.9	27.1
HPV Positive (months)	102.0	7.6	87.1	116.8	106.0	7.7	90.8	121.2
Overall (months)	**68.8**	**4.2**	**60.6**	**77.0**	**73.0**	**5.6**	**61.9**	**84.0**

Figure 1.4: Overall mean and median survival of OPC patients within GSTFT. P<0.001 Log Rank (Mantel-Cox) (S Thavaraj, 2019)

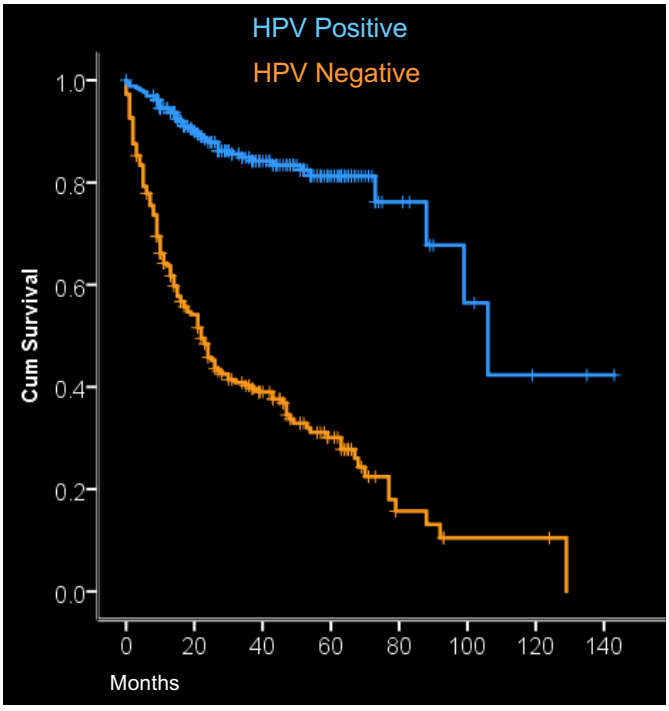


Figure 1.5: Overall survival of HPV positive and HPV negative patients at GSTFT (S Thavaraj, 2019).

Chapter 2

Introduction B: Oropharyngeal cancer and osteoradionecrosis

2.1 Osteoradionecrosis

Osteoradionecrosis (ORN) is a late effect of RT commonly but not exclusively associated with HNC (Wali et al., 2019). It was first reported by Reguad in 1922 (Reguad, 1922) and almost a century later continues to be regularly seen (Gadiwalla & Patel, 2018). Though preventive interventions have been employed the condition still occurs with no established treatment modality guaranteeing cure.

In the past two decades the introduction of novel RT targeted systems via IMRT was hypothesized to reduce or even eliminate ORN (Ben-David et al., 2007). Simultaneously, the demographics of HNC patient has seen a dramatic change with rise of OPC (Chaturvedi et al., 2013; Mehanna. et al., 2016). These 2 events have been arguably implicit in sustaining or even increasing the incidence of ORN now reported in the current era.

In brief, there is limited understanding regarding ORN, in particular with such substantial demographical change in HNC. Hence, much of the information and knowledge regarding ORN is historic, out-dated, and non-transferrable to a changing target population. This lack of knowledge extends to the pre- and post-RT phase of care and including the period leading up to ORN. These key phases in the patient pathway are likely to provide valuable information about the development of ORN and are investigated in this thesis.

2.2 Definition

Though ORN is a well-recognised condition no international agreed definition exists. A number of definitions have been proposed. Figure 2.1 presents the most commonly used definitions over the past 50 years.

Two major criticism of all the definitions is the lack of consideration for non-exposed and maxillary ORN which are both recognised in medication related osteonecrosis of the jaw (AAOMS, 2014). Both these types of ORN are routinely seen within our dedicated 'Bone & Necrosis Clinic' within the Institute. The lack of acknowledgement of these variant types of ORN further reflects the lack of understanding of this condition and processes leading up to its occurrence.

Author	Definition
(Beumer III et al., 1972)	When bone in the radiation field was exposed for at least 2 months in the absence of local neoplastic disease
(R. E. Marx, 1983)	An area greater than 1 cm of exposed bone in a field of irradiation that had failed to show any evidence of healing for at least 6 months
(Epstein et al., 1992)	An ulceration of the mucous membrane with exposure of necrotic bone
(Harris, 1992)	Irradiated bone exposed through the overlying skin or mucosa, persisting without healing for 3 months in the absence of tumour recurrence
(Hutchinson, 1996)	An area of exposed bone (mandible) present for longer than 2 months in a previously irradiated field, in the absence of recurrent tumour

Figure 2.1: Series of definition that have been commonly cited in the literature when referring to ORN

2.3 Osteoradionecrosis incidence

2.3.1 Head and neck cancer

Historically, in the pre-novel RT (3DCRT/IMRT) era the incidence of ORN in the HNC irradiated population was estimated to be 4.74 - 37.5% (Bedwinek et al., 1976; Daly et al., 1972; Grant & Fletcher, 1966; MacComb, 1962; Morrish Jr et al., 1981; Murray et al., 1980; Reuther et al., 2003; Watson, 1939; Withers et al., 1995). A systematic review calculated the base risk rate to be 2% increasing with additional risk factors (Syed Nabil & Samman, 2012). Though predicted to reduce following IMRT the incidence continues to be reported in substantial numbers ranging from 0.84 – 25.5% (Figure 2.2). Within our own institution (GSTFT) we have identified an ORN rate of 5.5% (De Felice et al., 2016).

However, assessing ORN based on a generic HNC diagnosis inevitably provides a wide incidence range due to the population bias within the group. Some HNC sub-sites are highly unlikely to experience ORN while others have an elevated risk. Therefore, pooling all HNC patients under a broad umbrella is likely to dilute the figures.

Author	No of patients	RT System	% ORN
(Studer et al., 2006)	73	IMRT	1.4
(Ben-David et al., 2007)	176	IMRT	0.0
(Gomez et al., 2011)	168	IMRT	1.2
(Montejo et al., 2011)	43	IMRT	2.3
(Nguyen et al., 2012)	83	IMRT	1.2
(Gevorgyan et al., 2013)	1575	3D-CRT IMRT	0.8
(Duarte et al., 2014)	158	3D-CRT(63%) IMRT (37%)	6.3
(De Felice et al., 2016)	653	3D-CRT(11%) IMRT (89%)	5.5
(Raguse et al., 2016)	149	3D-CRT(70%) IMRT (30%)	25.5
(Studer et al., 2016)	531	IMRT	7.0
(Kuhnt et al., 2016)	776	3D-CRT IMRT	6.6
(Aarup-Kristensen et al., 2019)	1224	3D-CRT IMRT	4.6

Figure 2.2: Incidence of ORN in HNC in the current era of novel RT delivery systems. Only large cohort studies were included.

2.3.2 Sub-site specific

The incidence of ORN is best appreciated when considering the primary HNC sub-site. Traditionally, OCC and nasopharyngeal cancer (NPC) have been accepted as possessing an elevated risk. Understandably this was due to RT delivery directly to or in immediate proximity to the jaws. The introduction of targeted RT was expected to reduce this risk (Ben-David et al., 2007), yet both sites continue to experience this complication and simultaneously OPC has emerged as another vulnerable sub-site for ORN. Interestingly a recent study reported no reduction in ORN rates in OPC patients even when comparing IMRT versus 3DCRT, both novel targeted RT systems (Maesschalck et al., 2016). This was in contrast to Tsai et al who did show a reduction with IMRT but still reported an incidence of 6% in OPC cohort (Tsai et al., 2013).

When assessing OPC alone in the new RT era, large cohort studies have shown an ORN rate ranging from 3.8 – 11% (Figure 2.3). Within our institution the overall ORN was 10.5% (n= 38/361) (Vinod Patel, 2019) and almost identical to Maesschalck et al (Maesschalck et al., 2016). Considering these are all large cohort studies, combining them suggests an overall risk of 5.9% in 4267 patients. Compared to the general HNC population excluding Raguse et al (Raguse et al., 2016) as a clear outlier, the overall risk is 3.4% in 5460 patients. Furthermore, the OPC rate appeared higher compared to many of the other sub-sites listed in figure 2.3.

However, these figures can be deceiving and provide little overall value, particularly, when trying to understand disease pattern. Caparotti et al presented mandibular ORN rates in OPC incrementally, reporting 3% at year 1, 5% at year 3 and 7% at year 5 (Caparrotti et al., 2017). In comparison, based on these time points our OPC cohort had an ORN rate of 4.7%, 9.4% and 10.5% respectively (Vinod Patel, 2019). Hence, the role of incremental time increase is an important factor in ORN and in OPC which is often overlooked or ignored.

Author	No of patients	Primary	RT System	% ORN
(Huang et al., 2008)	71	OPC	IMRT	1.4
(Avraham Eisbruch et al., 2010)	69	OPC	IMRT	6.0
(Setton et al., 2012)	442	OPC	IMRT	0.0
(Tsai et al., 2013)	402	OPC	3D-CRT (17%) IMRT (83%)	7.5
(Maesschalck et al., 2016)	234	OPC	3D-CRT (62%) IMRT (38%)	11.0
(A. Owosho et al., 2017)	724	OPC	IMRT	4.4
(Caparrotti et al., 2017)	1196	OPC	IMRT	6.0
(Wencheng Zhang et al., 2017)	584	OPC	IMRT (91%) IMPT (9%)	8.0
(Moon et al., 2017)	184	OPC	3D-CRT (11%) IMRT (89%)	3.8
(Vinod Patel, 2019)	361	OPC	IMRT	10.5
Other head and neck sub-sites				
(Tucker et al., 2016)	172	Salivary gland	IMRT	4.0
(Chen et al., 2016)	1692	OCC	IMRT	6.2
(A. Owosho et al., 2017)	299	OCC	IMRT	4.0
(Moon et al., 2017)	68	OCC	3D-CRT (11%) IMRT (89%)	7.0
(See Toh et al., 2018)	231	NPC	IMRT	2.6

Figure 2.3: Incidence of ORN in the novel RT delivery systems based upon specific HNC sub-sites

2.4 Classification

There is no agreed consensus on how to classify ORN and many still believe the condition only to affect the mandible hence excluding the maxilla from the grading system. A literature review to determine the various proposed classification showed there are a total of 18 classifications (Vinod Patel, 2016) with many of them explicitly focusing only on the mandible. Within our institute the ORN registry (2014 – to date) has recorded 31 cases of maxillary ORN out of a total of 220 cases. The inability to classify maxillary disease yet again highlights the limited and poor knowledge of ORN and the process leading to it.

2.5 Pathophysiology

The disagreement regarding definition and classification extends to the pathophysiology. The first accepted theory was proposed by Watson & Scarborough based on 3 critical factors of exposure to RT above a critical dose, local injury and infection leading to ORN (Watson, 1939). In 1970 Meyer extended this theory and proposed the term 'radiation induced osteomyelitis' suggesting the pathophysiology of radiation – trauma – infection (Meyer, 1970).

A decade later in 1983, Marx proposed a new theory realizing that in 35% of ORN cases no trauma was evident (R. E. Marx, 1983). He suggested that the affected tissue was burdened by hypoxia, hypocellularity and hypovascularity (3H theory) (R. E. Marx, 1983). Marx concluded that: *"ORN is not a primary infection of irradiated bone, but a complex metabolic and homeostatic deficiency of tissue that is created by radiation-induced cellular injury; micro-organisms play only a contaminating role in ORN; and trauma may or may not be an initiating factor"* (R. E. Marx, 1983).

2.5.1 Radiation induced fibrosis and atrophy

While some believe the 3H theory (R. E. Marx, 1983) to be valid others have moved to the latest theory of radiation induced fibrosis (RIF) and atrophy proposed by Delanian & Lefaix (S Delanian & Lefaix, 2004). RIF can be divided into four key clinical milestones: pre-fibrosis, established fibrosis, late fibrosis and atrophy/necrosis (S Delanian & Lefaix, 2004). As a consequence of RT, patients will experience some degree of fibrosis and the key factors driving this event remain the site receiving radiation as well as the associated dose. It is well recognised that fibrosis has a predilection for breast, skin, small bowel, lung, kidney and liver (Sylvie Delanian & Lefaix, 2007). Histopathological RIF is considered under 3 main categories: pre-fibrotic inflammatory phase, constitutive fibrotic cellular phase and late fibro-atrophic phase. These phases are progressive and occur over several years (Sylvie Delanian & Lefaix, 2007).

Though this theory is becoming popularised, limited information is present in either animal or human studies assessing these changes in the hard and soft tissue of oral cavity particularly in the current IMRT era.

2.6 Risk factors

A systematic review (Syed Nabil & Samman, 2012) found risk factors for ORN can be divided into 3 broad categories of oncological, dental and patient factors. However, this review lacked ORN cases related to IMRT and 3DCRT studies and did not recognise maxillary ORN.

Internal assessment of 197 ORN patients at GSTFT (our study cohort) (November 2014 – June 2019) identified 115 cases related to IMRT (58.4%). Considering only IMRT related ORN, the following features were observed: males (84/115, 73.0%); <60 years of age (53/115, 46.1%); current smokers (51/115, 44.3%); TNM7 Stage IV (82/115, 71.3%); OPC cases (61/115, 53.0%); chemo-RT treatment (54/115, 47.0%), radiation dose 55-65Gy (79/115, 68.7%); induced causes such as dental surgery (83/115, 72.2%) and mandible site (97/115, 84.3%) were the most commonly populated sub-groups.

2.6.1 Oncological related

2.6.1.1 Sub-site

Certain tumour sub-sites are an integral risk factor for ORN simply based on their proximity to the jaws. OCC and OPC are commonly associated with the mandible (A. Owosho et al., 2017) while tumours of nasopharyngeal or sinonasal origin demonstrate a higher incidence of ORN in the maxilla (Cheng et al., 2006). However, ORN is not limited to these two areas. A recent mini-case series highlighted that even with IMRT, ORN can occur to the wider maxillofacial skeleton such as the hyoid bone and temporal bones (Wali et al., 2019).

As indicated above some tumour sub-sites have been associated with ORN, but OPC distinguishes itself with a skewed leniency towards ORN. This was highlighted by Caparrotti et al drawing attention to the fact that patients are still at risk of developing ORN for many years after their treatment compounded by the fact–that HPV positive patients have high survivorship (Caparrotti et al., 2017). This pattern is mimicked in our own patient series (Figure 2.4) with a total of 38/361 patients developing ORN over a period of 5 years (Vinod Patel, 2019).

Review year	Number of patients	Number of new patients developing ORN
1	361	17
2	313	13
3	231	4
4	176	2
5 +	129	2

Figure 2.4: Number of ORN patients in post-RT OPC per subsequent review year at GSTFT (2011-2017) (Vinod Patel, 2019).

2.6.1.2 Radiotherapy

A systematic review (Syed Nabil & Samman, 2012) assessing various radiation schedules including different daily dose rates and treatment time identified conflicting results. However, total RT dose has been identified as a significant factor. Chang et al stated radiation doses >70 Gy were predictive of ORN (Chang et al., 2007) while Lee et al examined the dose-effect relationship for mandibular ORN in 198 patients with OCC, OPC and radiation doses above biologically equivalent values of 102.6 Gy were significantly associated with the development of ORN (J. Lee et al., 2009).

Owosho et al in an attempt to find the threshold dose for ORN induction compared the amount of radiation received via IMRT in ORN sites as compared to non-ORN sites in the same patient in their studies and found that ORN affected sites had been exposed to significantly more radiation (A. Owosho et al., 2017). In their study 96% of ORN sites in their patients had been exposed to radiation exceeding 60Gy (A. Owosho et al., 2017). Thorn et al also had the exact same results with only 3/80 ORN patients exposed to <60Gy radiation (J. Thorn et al., 2000). Kojima et al found the significant threshold dose to be lower at 50 Gy (Kojima et al., 2017). Though the threshold doses have been proposed and OPC patients identified as vulnerable to ORN the doses received to the jaw in this group using IMRT has not been well detailed.

The current research project builds on our preliminary work showing the changing pattern of ORN following retrospective analysis of data between the period of 2008-2015 at GSTFT (Haria et al., 2016). This range was ideal as it covered an era of pre-IMRT (2008 – 2011) as well as IMRT (2011 – 2015). A total of 125 patients had presented with ORN within this 7-year period with OPC (46.4 %, 58 patients) being the most common. From

the cohort, 65 (52%) patients were treated in the pre-IMRT era and 60 patients (48%) with IMRT.

2.6.1.3 Chemotherapy

Chemotherapy used in combination with RT seems to increase the risk of developing ORN (Sasahara et al., 2014) when compared to RT alone. A systematic review by Nabil & Samman found 5 studies supporting this view (Syed Nabil & Samman, 2012). It is estimated that the biological effects of concurrent chemotherapy on the tissue is equivalent to the addition of receiving a further 10 Gy of radiation (Kasibhatla et al., 2007).

The use of chemotherapy is increasingly used in the management of OPC and such been proposed as a significant reason for the rise of ORN in this HNC sub-site.

2.6.2 Dental related

The dentition in irradiated HNC patients is often considered the biggest risk factor and the main vulnerability for ORN. It is on this basis that it has become a mandatory requirement for all HNC patients due to receive RT to have a dental assessment prior (NICE, 2004; RCS, 2019; RD-UK, 2016). Though this intervention has seen a reduction in ORN rates from the historic figure of 37.5% a pre-RT dental review has not led to elimination of ORN.

In recent times it has been proposed that an ORN rate of <1% can be achieved with use of aggressive dental care in conjunction with IMRT (Studer et al., 2006). However, these figures have not consistently been replicated and 'aggressive dental treatment' has been interpreted in varying ways. With a changing landscape of HNC tumour groups and the use of IMRT the subsequent change to the presenting dental status has not been assessed. Dental oncologists have continued to employ traditional practices such as mass pre-RT dental extractions, particularly of molars in high dose areas in an attempt to avoid ORN and there is now controversy and debate on whether these practices need re-evaluating.

2.6.2.1 Pre-radiotherapy dental extractions

It has been long recommended that prior to commencing RT, patients should have a dental assessment. Guidance (RCS, 2019) suggests consideration towards achieving dental fitness via the removal of poor prognosis teeth and those with foci of dental infection. However, the guidance is not detailed and prescriptive and therefore has been

interpreted differently by both general dentist and dental specialists (dental oncologists). A spectrum of treatment has been seen ranging from a conservative approach with retention of periodontally involved teeth to the other extreme of prophylactic disease-free dental extractions due to their presence in regions due to receive high RT doses with the potential to cause ORN. Neither appear to have eliminated ORN from occurring.

Interestingly, a number of studies (Figure 2. 5) have focused on OPC and pre-RT extraction leading to ORN possibly due to the ironic phenomenon. Due to variant practices of dental oncologists in the pre-RT phase it remains impossible to hypothesize on the dental status of OPC. However, there appears to be an importance attached by dental oncologist to this tumour group regarding both their dentition and expected RT doses to the teeth and jaws. Minimal information exists in the literature regarding these specifics to OPC and IMRT.

Author & Year	Study total	RT Type	Primary Tumour	% ORN in pre-RT extraction
(Chang et al., 2007)	413	EBT	OPC	15.0
(Tsai et al., 2013)	402	3DCRT IMRT	OPC	2.2
(N. M. Beech et al., 2017)	109	Not stated	OPC	2.0
(Muraki et al., 2019)	67	Not stated	HNC	7.0
(Aarup-Kristensen et al., 2019)	1224	3DCRT IMRT	HNC	3.5
(Vinod Patel, 2019)	361	IMRT	OPC	8.0

Figure 2.5 Studies in the literature reporting on pre-RT dental extractions and the incidence of ORN

2.6.2.2 Post-radiotherapy dental extractions

Post-RT dental extraction remains the intervention with the greatest threat for the development of ORN. The treatment induced oral environment of xerostomia, trismus, high calorific liquid food supplements and chronic mucositis all lead to sub-optimal oral hygiene and facilitate the induction of radiation caries. This well-known scenario has

driven the practice of prophylactic dental extractions at the pre-RT phase which in the OPC group has proven to be unacceptable to the patients.

Limited information is known about post-RT dental extraction in the IMRT phase of treatment however as identified earlier (2.3 osteoradionecrosis incidence), the ORN rate is increasing in the novel radiation era. Most studies have either limited patient numbers or amalgamate all HNC together therefore providing unreliable post-extraction ORN rates for sub-groups of patients. RT dose received at the extraction site is a significant risk factor with a maximum mandibular dose >70 Gy and a mean mandibular dose >40 Gy associated with increased subsequent dental events and extractions after IMRT (Gomez et al., 2011).

Assessment of our IMRT related ORN cohort found 83/115 patients developed the complication from induced causes such as dental extraction as opposed to spontaneous necrosis. Within this cohort, OPC (61/115) was the dominating HNC sub-site. Hence, we subsequently assessed all new OPC prospectively in our institute to determine when and how they developed ORN (Figure 2.6). Interestingly, we identified that the induced causes such as dental extraction had a sustained rate of approximately 1-2% annually whereas spontaneous ORN was elevated in the first 2 years and then dramatically fell to <1% subsequently (Figure 2.6)

	Overall		**Induced cause**		**Spontaneous cause**	
Review Year	**Number of ORN**	**% of ORN/year**	**Number of ORN**	**% of ORN**	**Number of ORN**	**% of ORN**
1	17/361	4.7	6/361	1.7	11/361	3.0
2	13/313	4.2	4/313	1.3	9/313	2.9
3	4/231	1.7	3/231	1.3	1/231	0.4
4	2/176	1.1	2/176	1.1	0/176	0.0
5+	2/129	1.6	1/129	0.8	1/129	0.8

Figure 2.6: Number of OPC patients reviewed in subsequent years and the ORN rate from induced and spontaneous causes at GSTFT (2011-2017) (Vinod Patel, 2019).

In the era prior to IMRT a systematic review suggested the estimated incidence of ORN after tooth extraction in irradiated patients to be 7% (S Nabil & Samman, 2011). This rate

reduces with prophylactic interventions such as antibiotics (6%) and hyperbaric oxygen therapy (HBOT) (4%) (S Nabil & Samman, 2011). However, a recent randomised control trial (HOPON) comparing antibiotics versus HBOT did not find significant difference between the two interventions (Shaw et al., 2019). In contrast, pilot results from the prophylactic use of pentoxifylline and vitamin e (PVe) suggested a lower ORN rate of only 1.2% (V Patel et al., 2016a). When comparing ORN rate per tooth the systematic review (S Nabil & Samman, 2011) proposed a 2% rate while the use of prophylaxis PVe was 0.26% (V Patel et al., 2016a).

2.6.3 Patient related

Several patient related factors have been reported in the literature in relation to ORN highlighting the condition being of multi-factorial origin.

Reuther et al found that men are 3 times more likely to develop ORN (Reuther et al., 2003) while Khunt et al stated the average age of ORN was 55 ± 10.1 years following novel RT delivery (Kuhnt et al., 2016). Interestingly, these peak demographics overlap with the presenting profile of OPC patient.

Smoking has been consistently identified as major risk factor for ORN. Considering the HNC population has a relatively high proportion of smokers it is commonly cited as a major risk factor (Tsai et al., 2013). Patients who continued to smoke during radiation treatment had a 32% increased risk for the development of ORN (Zevallos et al., 2009). Alcohol has less consistently been identified as a risk factor. However, Owosho et al found in the IMRT era in both OCC and OPC patients, 75% of patients continue to drink alcohol and found that they were 3.22 more times likely to develop ORN (A. Owosho et al., 2017).

2.7 Management

The management of ORN has naturally been dictated by the evolving pathophysiological theories proposed over the decades. However, no treatments have been able to claim guaranteed cure. Though some authors report high success rates for a specific treatment these have not been consistently repeated by others or over time. The inability to confidently manage ORN returns back to lack of knowledge of the fundamental pathophysiological mechanisms driving this condition.

2.7.1 Antibiotics

Meyer's 'radiation induced osteomyelitis' theory led to the basis for both antibiotics and surgery as treatment modalities (Meyer, 1970). Though this theory has been superseded both approaches continue to be a mainstay strategy.

The practice of antibiotics prescription for ORN has continued because the necrotic bone is susceptible to secondary infection. A wide range of antibiotics have been reported in the literature with no one drug of specific advantage for ORN. The clinical protocol employed involves a broad-spectrum antimicrobial in the acute setting and if necessary, switching to low dose long term antibiotic in chronic infection. In such cases a tetracycline-based antibiotic is the preferred choice as it is chelated by the calcium of the bone and incorporated into the bone crystals (Coffin, 1983).

The use of long-term antibiotics is likely to lead to resistance and unlikely to lead to cure. Both are unfavourable outcomes in OPC patients, particularly in the HPV positive group who have high survivorship.

2.7.2 Hyperbaric oxygen therapy

Mainous et al proposed the use of HBOT (Mainous et al., 1973) but it was Marx's endorsement following the 3H theory which popularized its use (R. E. Marx, 1983). Simply used alone the results are poor and the recommendation is to use it as an adjunct to surgery. The objective is to prime and optimize the compromised surgical site for healing. The use of HBOT pre-surgery is still routinely used in the USA but has slowly fallen out of favour in the UK particular after the recent publication of the HOPON study (Shaw et al., 2019). This well conducted study failed to show its superiority when used in RT patients. A recently published Cochrane review of Late Radiation Tissue Injury in 3 trials, focused on achieving complete mucosal cover in ORN patients. There was a significantly improved probability of attaining mucosal cover (Bennett et al., 2016).

2.7.3 Surgery

Under the umbrella of surgery, a multitude of procedures exists and range from minimal to radical. Commonly, these include sequestrectomy, saucerisation, debridement and

resection with or without vascularized free tissue transfer. In the latter, a major concern in OPC patients is worsening their pre-existing dysphagia and trismus as a result of such radical surgery. Outcomes for these procedures widely vary and when all other strategies fail surgery remains the default option if not already considered as the primary option.

A study (V. Patel et al., 2017) focusing only on severe ORN requiring resection identified the huge resource and financial implications in surgically managing these cases. However, surgery will continue to be a realistic option as success can be achieved in selected cases with the advantage of providing dental rehabilitation.

2.7.4 Pentoxifylline – Tocopherol – Clodronate

The most recent radiation induced fibrosis theory has led to a novel approach via pharmacological management (S Delanian & Lefaix, 2004). Initially a combination of two medications; pentoxifylline and tocopherol (S. Delanian et al., 2003) were used which were then supplemented with clodronate (S. Delanian et al., 2011; S. Delanian & Lefaix, 2002; Robard et al., 2014). When all three are used in conjunction they are often referred to as PENTOCLO. In our institution (V Patel et al., 2016b), this treatment strategy has achieved results equating that of Delanian et al (S. Delanian et al., 2011) notably healing > 50% of cases. A recent systematic review found the combination of pentoxifylline plus tocopherol with or without clodronate to be effective for the treatment of mandibular ORN (Martos-Fernández et al., 2018). One major limitation is their availability only in tablet formation which impacts on HNC patients with swallow restriction or those tube fed (nasogatric or percutaneous endoscopic gastrotomy). These drugs have been converted to a liquid preparation but their efficacy, dose and regime in this formulation is yet to be validated (V Patel et al., 2018).

Pentoxifylline is a methylxanthine derivative licensed for use in peripheral vascular disease. It is only available in tablet (modified release) formulation in the UK. It has 4 main properties. It increases erythrocyte deformability (Honess et al., 1993), decreases blood viscosity (Honess et al., 1993), inhibits human dermal fibroblast proliferation and extracellular matrix production (Berman & Duncan, 1990) and finally increases collagenase activity (Berman & Duncan, 1990).

Alpha-tocopherol often known as vitamin E is an antioxidant and commonly available in both suspension and liquid capsules. It protects membrane phospholipids from oxidative

damage by scavenging reactive oxygen species generated during oxidative stress (Packer et al., 2001). Additional properties include inhibition of protein kinase C with consequent inhibition of platelet aggregation, nitric oxide production in endothelial cells, and superoxide production in neutrophils and macrophages (Packer et al., 2001).

Clodronate is a second-generation bisphosphonate that inhibits bone resorption by reducing osteoclastic activity but equally act directly on osteoblastic cells by increasing formation of bone and reducing proliferation of fibroblasts (Fast et al., 1978; Fromigue & Body, 2002). Its addition primarily leads to both sequestration of dead bone and formation of new bone but also expedites the efficacy of pentoxifylline and tocopherol (S. Delanian et al., 2011).

The success of this regime validates the current pathophysiology theory of ORN. Historically, it has seen successful in reducing radiation induced fibrosis (RIF) across the body (V Patel & M McGurk, 2017). In dental cases, its use has now extended successfully from therapeutic intervention to prophylaxis in irradiated patients undergoing dental extractions (V Patel et al., 2016a). Its preventive potential is now being tested in OPC patients by prescribing the combination immediately after RT (ISRCTN, 2019) with the endpoint being reduction in ORN, trismus and dysphagia.

2.8 New and evolving future challenges

There are significant challenges ahead for clinicians managing these patients when balancing efforts to cure the patient of cancer while restricting post RT complications.

The current rising epidemic of OPC produces an enlarging pool of surviving patients with radiation induced risks. No measures are currently being taken to reduce or counterbalance these risks as the dynamics driving this disorder are not well understood. It is acknowledged the OPC is on the increase and this group carry a higher risk for ORN. Apart from this little is known. Dental factors and RT doses are pivotal to ORN. A thorough assessment of the OPC cancer journey from diagnosis to ORN should be evaluated with these factors in mind.

Integral to this evaluation is the standard of the dentition prior to RT. Information from large IMRT cohort studies does not exist. Consequently, the dental status of OPC patients in the current era is unknown but will need context to have meaning. The dental status of

other sub-sites and non-HNC patients need to act as a comparator. Only with this knowledge can one produce an appropriate pre-RT treatment plan. The importance of teeth to QoL is well known as is the detrimental impact of mass pre-RT extractions (N. Beech et al., 2016). This aspect of dental planning has not been considered in OPC patients despite their youth and favourable survival.

Secondly, the impact of RT on the teeth based on detailed dosing schedules needs exploring. Currently, pre-RT dental treatment planning takes place with minimal information of the doses of radiation each dental unit receives. The 'at risk' teeth are unknown so specific preventive measures cannot be target on them. Dental practitioners remain oblivious to the dental variation in RT doses and prolonged survivorship in OPC patients. This is pertinent to treatment planning.

Thirdly, with the advent of RIF theory, the impact of RT on the hard and soft tissues of the jaw need evaluating with time and RT dose in mind. Once again, minimal information is known specific to OPC and IMRT. Currently this information does not exist with respect to OPC patients and IMRT treatment. These points are addressed in the following chapters.

Chapter 3

The dental status of head and neck cancer patients in general prior to commencing intensity modulated radiation treatment

3.1 Introduction

It is universally accepted that the dentition of HNC patients should be assessed in preparation for radiotherapy (RT) (NICE, 2004). In the UK, guidance is offered via the Royal College of Surgeons (RCS, 2019) and by Restorative Dentistry UK (RD-UK, 2016). The guidance highlights major dental issues a patient may face and offers advice on both preventing and managing these issues if they present. The underlying driver for this intervention is a generally accepted stereotype that HNC patients have sub-optimal dental status, which then subsequently places them at risk of ORN. A case control study by Tezal et al strengthened this narrative (Tezal et al., 2013). Compared to non-HNC patients, their presenting dentition was more periodontally involved with less teeth overall (Tezal et al., 2013). This was equally true in the UK with Critchlow et al reporting extensive periodontal disease with only 64% of patients having 21 or more teeth and high decayed/missing/filled teeth (DMFT) scores (Critchlow et al., 2014). This is unsurprising considering Frydrych et al reported most HNC patients had not visited a dentist in the year preceding their cancer diagnosis and the mean time to the last visit was 5.6 years (Frydrych & Slack-Smith, 2011). These findings reflect trends in the HNC group as a whole as having poor dental awareness with neglected oral hygiene, irregular dental attendance and a preference towards dental extraction rather than restoration of the dentition. Essentially, teeth do not appear to be a priority in this group. Traditionally the HNC patient had a history of excess smoking and alcohol consumption and were easy to stereotype. The adverse effect of these factors on dental homeostasis is well known particularly regarding the association of periodontal disease to smoking (Axelsson et al., 1998) and the habit closely associated with oral cavity, oropharyngeal and laryngeal cancer (Kojima et al., 2017; Tezal et al., 2013). Excessive alcohol adversely impacts oral hygiene which translates into poor DMFT scores and periodontal disease (Manicone et al., 2017). The effects are exacerbated when both habits are encountered in the same individual (Manicone et al., 2017) particularly when the individual's lifestyle does not value oral health.

Factors contributing to the success and survival of HNC patients are routinely assessed via demographics such as gender, age and smoking status and at the tumour level; site, size and nodal involvement. In developing a dental oncology treatment plan for an individual it is unclear whether these factors have any association or relevance with dental status. These have not been mapped onto indices that have an association with outcome such as gender, age, smoking history, HPV status and tumour staging. However,

these factors are considered either consciously or sub-consciously in clinical decision-making pre-RT as judgments to whether certain teeth will survive based on the severity of the tumour or whether they will receive a heightened dose of radiation and the impact of this. The information we currently accept at this level is often anecdotal and requires either validating or excusing if incorrect. Hence, the decision process to achieve an optimal dental status is multifactorial and a complex problem.

The principal dilemma is whether to retain healthy teeth that may pose a risk of inducing ORN in the future. Balanced against the risk of ORN is the evidence that greater than eight pre-RT dental extractions, HPV negative status, female gender, and positive smoking status were associated with statistically significant reduced QoL (N. Beech et al., 2016). There is no scientific evidence to guide treatment and currently management falls to expert opinion (RD-UK, 2016). Paradoxically, recent evidence suggests pre-RT dental extractions may increase the risk of ORN (N. M. Beech et al., 2017).

The conundrum facing the dental oncologist is that multiple tooth extraction leads to a reduced QoL while retention of compromised teeth adds to the risk of ORN. A social factor not commonly appreciated is that soon after being informed of their malignant diagnosis, patients are further informed of the imminent need to lose teeth, often multiple and at times healthy or unrestored. This can lead to negative emotional impact (Clough et al., 2018) in an individual already in a heightened emotive state.

The past decade has seen significant changes in both the demographics of the HNC patient and their dental health. Within oncology, there has been a rise in OPC while simultaneously a decrease in oral cavity cancer (OCC) (Chaturvedi et al., 2013) and with it a change in patient stereotype. Furthermore, the past decade has seen the introduction and routine use of a novel RT regimes; notably intensity modulated radiation therapy (IMRT). The vision was it would reduce or even eliminate RT-related effects such as ORN. Over the same period, the last Adult Dental Health Survey (ADHS) in 2009 confirmed that the nation's dental health has been improving with every decade (ADHS, 2009), but it remains unclear whether this improvement extends to the modern cohorts of HNC patients.

The current study is an observational study aiming to assess the dental status of HNC patients at the pre-RT phase. It first relates the dental health of HNC patients in general to the overall population to determine whether their dental status still lags behind the

norm. It then focuses on the dental status of the 3 leading sub-sites of HNC (DAHNO, 2014); (laryngeal cancer (LC), oral cavity cancer (OCC) and oropharyngeal cancer (OPC)).

3.2 Methodology

3.2.1 Ethics

19/EE/0224 - Dental status, radiotherapy doses and subsequent implications in head and neck cancer patients - A retrospective cohort study

3.2.2 Data Collection

The electronic appointments log for Guys & St Thomas' HNC pre-RT dental assessment clinic was retrieved for the period of March 2011 until December 2017. The log commenced at March 2011 following the introduction and routine use of IMRT for all HNC patients at the hospital. Internal yearly audit over the past 5 years has shown that 95-98% of HN RT patients had a dental assessment prior to commencing treatment. Reasons for missed assessment were failure to attend, urgent initiation of RT or patient infirmity. These patients were not captured in the current database.

The electronic out-patient appointments system yielded a total of 1360 new patient appointment. Inclusion criteria included biopsy proven HNC with curative intent requiring RT. A total of 474 patients were excluded (non-squamous cell HNC, distant metastases, RT with palliative intent, previous history of HNC). Edentulous patients were also excluded as not all these patients are routinely sent for pre-RT dental review. Following exclusions, a total of 886 patients were included in this retrospective study.

All data was collected using fixed options per category. The domains included patient demographics (gender, age, smoking status) and tumour demographics (sub-site, tumour size, nodal status, HPV status, overall staging) using multi-disciplinary meeting records. The dental status of each patient was collected using their presenting dental panoramic tomograph (DPT), which is used as a primary screening image for all patients at their pre-RT dental assessment. Under the umbrella of dental status, data was collected under five domains; teeth present, carious or restored teeth, DMFT score and severity grading of horizontal bone loss (HBL). All of these excluded the presence and number of third molars for which the data was collected as a separate domain. Severity of HBL was graded as nil (0), mild (1), moderate (2), severe (3) for all 6 sextants with the sum providing an overall

score for the patient. The total score provided a generalised severity grading (0: no generalised HBL, 1-6: mild, 7-12: moderate, 13-18: severe). The severity was judged on the basis of thirds along the tooth root length. This meant mild equated to a maximum of a third, moderate a maximum of two-thirds and severe beyond two-thirds HBL.

All data collectors were standardised by jointly carrying out data collection for the same 20 patients. In addition, the author VP audited the data by assessing 87 (10%) random patients to check for standardisation.

3.3 Statistical analysis

The sample and outcome data were summarised using descriptive statistics. The mean dental status based on gender was compared using unpaired t-test. The dental statuses of the remaining categories (age, smoking, tumour stage, nodal status, sub-site) were compared using one-way ANOVA. Significance was assumed at the 5% level, and analyses included the use of IBM SPSS Statistics for Windows, version 23.0 (IBM Corp).

3.4 Results

3.4.1 Global study population

3.4.1.1 Demographic breakdown

A total of 886 patients were evaluated with males (n = 641, 72%) heavily dominating the cohort. The most common age group was 55-64Y (n=295, 33%). Forty percent of patients were ex-smokers (n = 336, 37.9%) followed closely by current smokers (n = 321, 36%). Tumour demographics showed the OPC (n = 320, 36%), stage IV (n= 509, 57.4%), T2 size tumours (n = 257, 29%) and N2 nodal status (n = 416, 47%) were the most populated indices. Figure 3.1 provides the category breakdown.

Demographics	Subcategories (N)
Gender	Male (641)
	Female (245)
Age Group	16-24 (2)
	25-34 (18)
	35-44 (58)
	45-54 (217)
	55-64 (295)
	65-74 (215)
	75-84 (76)
	85+ (5)
Smoking Status	Current (321)
	Ex-smoker (336)
	Never (229)
Tumour Site	Paranasal & Sinus (25)
	Salivary Glands (62)
	Unknown Primary (49)
	Hypopharynx (24)
	Nasopharynx (39)
	Oral Cavity (154)
	Larynx (192)
	Oropharynx (320)
	Other Head & Neck (21)
Stage	I (76)
	II (110)
	III (135)
	IV (509)
Tumour Size	T0 (56)
	T1 (166)
	T2 (257)
	T3 (182)
	T4 (225)
Nodal Size	N0 (345)
	N1 (85)
	N2 (416)
	N3 (40)

Figure 3.1: All 886 patients sub-categorised. (Note: Stage category excludes T0 patients)

3.4.1.2 Dental status based on gender

The difference in dental status between genders is presented in figure 3.2. Both genders appeared to have on average a similar dental status with less than one tooth difference in all categories. However, the number of third molars was statistically significant ($p=0.0037$) with males presenting with a higher number compared to females.

3.4.1.3 Dental status based on age

Increasing age lead to a one-way trend of declining dental health in all criteria (Figure 3.3). Each category showed high significance ($p \leq 0.005$). Observationally, there was minimal difference in dental health from 65 years onwards in the various dental categories.

3.4.1.4 Dental status based on smoking status

Patients who actively smoked at the time of diagnosis had a considerably worse dental status than those patients who were either ex-smokers or never smoked (Figure 3.4). Smokers had significantly less teeth ($p=0.0104$) including third molars ($p=0.007$), higher DMFT scores ($p=0.0000$) and poorer horizontal bone loss scores ($p=0.0000$) than non- and ex-smokers.

3.4.1.5 Dental status based on oncological staging

Staging patients based on TNM7 showed no significant difference in patient's dental status (Figure 3.5). As the oncological staging increased so did the population of each group. A steady increase was evident from Stage I-III. However, Stage IV alone made up over half the patient cohort.

3.4.1.6 Dental status based on tumour size

A general inverse trend was evident for the mean number of carious/restored teeth as tumour size increased ($p=0.0050$). Horizontal bone loss was also weakly significant in association with tumour size ($p=0.0373$) however, no discernible trend was obvious. Both mean number of teeth present, and third molars decreased with increasing tumour size, but this was not seen to be significant (Figure 3.6).

3.4.1.7 Dental status based on nodal size

Increasing nodal status identified an increase in both mean number of teeth ($p=0.0437$) including third molars ($p=0.0195$). The trend was opposite to that evident for tumour size. A similar trend was also evident for carious/restored teeth and DMFT however these were not determined to be significant (Figure 3.7).

3.4.1.8 Dental status based on head and neck cancer sub-site

HNC patients were sub-divided based on standardized sub-site nomenclature (Figure 3.8). There was strong significance ($p \leq 0.004$) in dental status differences in all dental categories based on tumour sub-site. NPC followed by salivary gland and HNC (other) observationally had the best dentitions based upon most teeth and least dental disease. However, these sub-sites were essentially minority groups within the HNC cohort. Within the 3 most common sub-sites (OPC, LC, OCC), OPC patients had a better dentition based on more teeth and lower DMFT and horizontal bone loss score.

	Male (641)	Female (245)	p value
Teeth Present	20.1 (7.1)	19.5 (7.6)	**0.2703**
Caries/Restored	8.1 (5.1)	8.2 (5.4)	**0.7974**
DMFT	15.9 (7.1)	16.5 (7.1)	**0.2609**
Horizontal Bone Loss	7.5 (3.9)	7.3 (3.7)	**0.4889**
Third molars present	1.4 (1.4)	1.1 (1.3)	**0.0037*#**

Figure 3.2: Mean values for each dental domain with standard deviation in brackets based on gender. *Unpaired t-test with 95% confidence intervals with p values with those <0.05 marked by an *. Bonferroni correction (p<0.01) denoted as #*

	16-24 (2)	25-34 (18)	35-44 (58)	45-54 (217)	55-64 (295)	65-74 (215)	75-84 (76)	85+ (5)	p value
Teeth Present	28 (0)	26.3 (2.7)	24.4 (4.9)	22.3 (6.0)	19.7 (7.4)	17.5 (7.7)	16.0 (6.6)	17.0 (4.4)	**0.0000*#**
Caries/Restored	0 (0)	2.7 (3.1)	6.0 (4.6)	7.9 (4.8)	8.8 (5.5)	8.5 (5.5)	8.0 (4.3)	9.0 (2.2)	**0.0000*#**
DMFT	0 (0)	4.3 (4.4)	9.6 (7.4)	13.5 (6.9)	17.0 (6.3)	18.8 (5.9)	19.8 (5.1)	19.8 (3.8)	**0.0000*#**
Horizontal Bone Loss	1.5 (2.1)	3.8 (2.6)	6.1 (3.3)	7.0 (4.1)	7.8 (3.8)	7.7 (3.7)	8.0 (3.0)	10.6 (2.5)	**0.0000*#**
Third molars present	3.0 (1.0)	1.9 (1.6)	1.8 (1.6)	1.4 (1.5)	1.3 (1.4)	1.1 (1.2)	0.9 (1.1)	1.2 (1.3)	**0.0005*#**

Figure 3.3: Mean values for each dental domain with standard deviation in brackets based on age decades. *One-way ANOVA with 95% confidence intervals with p values with those <0.05 marked by an *. Bonferroni correction (p<0.01) denoted as #*

	Current (321)	Ex-smokers (336)	Never (229)	p value
Teeth Present	18.1 (7.8)	19.9 (7.1)	22.5 (5.9)	0.0104*
Caries/Restored	7.6 (5.3)	8.3 (4.8)	8.5 (5.2)	0.0814
DMFT	17.4 (7.1)	16.3 (6.8)	13.8 (7.1)	0.0000*#
Horizontal Bone Loss	8.4 (4.0)	7.5 (3.7)	5.9 (3.3)	0.0000*#
Third molars present	1.2 (1.4)	1.2 (1.3)	1.6 (1.4)	0.0007*#

Figure 3.4: Mean values for each dental domain with standard deviation in brackets based on smoking status. *One-way ANOVA with 95% confidence intervals with p values with those <0.05 marked by an *. Bonferroni correction (p<0.01) denoted as #*

	I (76)	II (112)	III (133)	IV (488)	p value
Teeth Present	19.0 (7.4)	19.0 (7.9)	19.5 (7.7)	20.2 (7.1)	**0.2732**
Caries/Restored	8.0 (5.3)	8.3 (5.0)	7.6 (5.5)	8.2 (5.1)	**0.6549**
DMFT	17.0 (7.2)	17.1 (7.5)	15.9 (7.2)	15.8 (7.0)	**0.2231**
Horizontal Bone Loss	7.3 (3.7)	7.5 (3.8)	7.9 (3.8)	7.3 (3.8)	**0.4331**
Third molars present	1.2 (1.2)	1.2 (1.3)	1.3 (1.4)	1.3 (1.4)	**0.8566**

Figure 3.5: Mean values for each dental domain with standard deviation in brackets based on TNM7 classification for staging (excludes T0s and head and neck cancer other). *One-way ANOVA with 95% confidence intervals with p values with those <0.05 marked by an *.*

	T0 (56)	T1 (166)	T2 (257)	T3 (182)	T4(225)	p value
Teeth Present	21.5 (6.5)	20.2 (7.6)	20.5 (7.1)	19.2 (7.2)	19.2 (7.5)	0.0763
Caries/Restoration	8.7 (5.9)	8.5 (5.1)	8.9 (5.3)	7.3 (4.9)	7.5 (5.3)	0.0050*#
DMFT	15.2 (7.0)	16.1 (7.1)	16.2 (7.2)	15.9 (6.8)	16.2 (7.4)	0.8951
Horizontal Bone Loss	7.4 (4.1)	6.7 (3.5)	7.4 (3.8)	8.0 (3.9)	7.5 (3.9)	0.0373*
Third Molars Present	1.5 (1.4)	1.2 (1.4)	1.3 (1.4)	1.3 (1.4)	1.3 (1.4)	0.7407

Figure 3.6: Mean values for each dental domain with standard deviation in brackets based on tumour stage classification. *One-way ANOVA with 95% confidence intervals with p values with those <0.05 marked by an *. Bonferroni correction (p<0.01) denoted as #*

	N0 (345)	N1 (85)	N2 (416)	N3 (40)	p value
Teeth Present	19.1 (7.4)	19.9 (8.3)	20.5 (6.9)	21.1 (7.0)	0.0437*
Caries/Restorations	7.7 (5.1)	8.4 (6.0)	8.5 (5.2)	7.4 (4.9)	0.1444
DMFT	16.5 (7.4)	16.2 (7.3)	15.8 (6.7)	14.3 (7.2)	0.2205
Horizontal Bone Loss	7.6 (3.9)	6.7 (3.3)	7.4 (3.9)	7.8 (3.7)	0.2466
Third Molars Present	1.2 (1.3)	1.4 (1.4)	1.3 (1.4)	1.9 (1.6)	0.0195*

Figure 3.7: Mean values for each dental domain with standard deviation in brackets based on nodal stage classification. *One-way ANOVA with 95% confidence intervals with p values with those <0.05 marked by an *. Bonferroni correction (p<0.01) denoted as #*

Tumour Site	Teeth present	Carious or restored teeth	DMFT	Horizontal bone loss	Third molar present
Paranasal and sinus (25)	19.0 (6.9)	7.9 (4.5)	17.0 (7.3)	7.6 (3.8)	1.0 (1.2)
Salivary Gland (62)	22.1 (6.7)	8.6 (5.1)	14.3 (7.1)	5.4 (3.1)	1.1 (1.2)
Unknown primary (49)	21.1 (6.6)	8.8 (6.1)	15.7 (7.1)	7.7 (4.2)	1.4 (1.3)
Hypopharynx (24)	19.5 (7.6)	7.8 (4.7)	16.2 (5.6)	8.0 (3.5)	1.0 (1.0)
Nasopharynx (39)	23.7 (4.9)	5.1 (4.8)	9.0 (6.8)	6.6 (3.6)	2.2 (1.6)
Oral Cavity (154)	18.3 (7.7)	7.3 (5.5)	16.8 (7.6)	7.4 (3.8)	1.3 (1.3)
Larynx (192)	17.2 (7.5)	7.6 (4.8)	18.3 (6.4)	8.7 (3.7)	1.1 (1.3)
Oropharyngeal (320)	21.1 (6.9)	9.0 (5.1)	15.7 (6.7)	7.2 (3.8)	1.4 (1.5)
Head & neck - other (21)	22.2 (5.7)	8.3 (6.0)	13.8 (7.0)	5.7 (4.2)	1.8 (1.7)
p value	**0.0000*#**	**0.0002*#**	**0.0000*#**	**0.0000*#**	**0.0004*#**

Figure 3.8: Mean values for each dental domain with standard deviation in brackets based on head and neck cancer sub-sites. *One-way ANOVA with 95% confidence intervals with p values with those <0.05 marked by an *. Bonferroni correction (p<0.01) denoted as #*

3.4.2 Sub-cohort population

3.4.2.1 Sub-cohort population demographics

Within the various HNC sub-site the 3 most populous regions were OPC (n=320, 48%), LC (n=192, 29%) or OCC (n=154, 23%). Jointly, these 3 sites accounted for over three quarters of the total HNC population (n= 666/886, 75.2%)

Males again dominated overall (n=491, 74%) and within each sub-site tumour group. The age range peaked at 55-64Y (n=227, 34%) with decades either side consisting of 166 patients (25%) each. There were a similar number of smokers (n = 259, 39%) and ex-smokers (n= 262, 39%). The disease was staged by both TNM7 & TNM8 criteria with stage IV disease recorded as 65% (n= 431) and 36% (n= 242) respectively. A breakdown of data is shown in Figure 3.9.

	OPC (n=320)	LC (n= 192)	OCC (n = 154)	Total
Gender				
Male	240	157	94	491
Female	80	35	60	175
Age				
25-34	2	0	6	8
35-44	17	11	7	35
45-54	97	30	39	166
55-64	123	62	42	227
65-74	66	55	45	166
75-84	15	32	13	60
85+	0	2	2	4
Smoking Status				
Current	106	104	49	259
Ex-smoker	131	76	55	262
Never	83	12	50	145
Staging (TNM 7)				
I	6	40	11	57
II	26	37	9	72
III	27	54	25	106
IV	261	61	109	431
Staging (TNM 8)				
I	26	40	11	77
II	150	37	9	196
III	68	54	25	147
IV	72	61	109	242

Figure 3.9: Current cohort of 666 patients sub-categorised via basic and oncological demographics. (TNM8 section totals 662 as 4/320 OPC patients were excluded with unknown HPV status therefore could not be re-staged)

3.4.2.2 Dental status based on gender

The pre-RT dental status of each sub-site was compared based on gender (Figure 3.10). Male OPC patients had on average more teeth on presentation ($p = 0.0000$), higher carious/restored teeth ($p=0.0018$), lower DMFT ($p=0.0004$), less HBL ($p=0.0000$) and higher third molars present ($p=0.0020$) than other sub-sites. There was no statistical significance in dental status in females for the various sub-sites.

3.4.2.3 Dental status based on age

Increasing age for all 3 sub-sites was inversely related (Figure 3.11) to the number of teeth present while DMFT scores increased. In the OPC group, the mean number of caries/restored teeth increased with age (p=0.0142). HBL severity score worsened with age in OCC patients (p=0.0024).

3.4.2.4 Dental status based on smoking status

A smoking habit was associated with low tooth retention. LC patients had the least number of teeth out of the 3 sub-sites for active smokers (p=0.0026) and ex-smokers (p=0.0011). Those that never smoked had the most teeth (p=0.0067). The LC ex-smokers' group, had the highest DMFT score (p=0.0054) and the most severe HBL score (p=0.0005). The non-smoking LC group had both higher tooth retention (p=0.0067) and the most carious/restored teeth (p=0.0007). Figure 3.12 shows a comparative assessment of all 3 tumour groups based on smoking status.

3.4.2.5 Dental status based on oncological staging (TNM7)

With advanced stage of disease in accordance with TNM7 the number of teeth present fell and caries/restoration increased respectively for stage III (p = 0.0037 & 0.0154) and stage IV (p= 0.0002 & 0.0064) across sub-sites Additionally, in stage IV the DMFT score (p=0.0184) and HBL (p=0.0004) were also significant (Figure 3.13).

3.4.2.6 Dental status based on oncological staging (TNM8)

Interestingly, when the patients were re-staged according to TNM8 dental significance based on tooth retention and DMFT shifted downwards to stage I (p=0.0011 & 0.0322), stage II (p=0.0005 & 0.0069) and stage III (p=0.0001 & 0.0175). In both staging systems, OPC patients had the most teeth present and the lowest DMFT. In contrast, in TNM8, LC patients had higher HBL scores for stage IV (p=0.0022) (Figure 3.14).

3.4.2.7 Dental status based on micro-sites

Dividing the 3 sub-sites into further specific tumour micro-sites did not reliable identify any major difference in dental status (Figure 3.15).

	Sub-site (N)	Male	p value		Sub-site	Female	p value
Teeth Present	*Larynx (157)*	17.2 (7.3)			*Larynx (35)*	17.5 (8.1)	
	Oral Cavity (94)	19.0 (7.4)	0.0000*†		*Oral Cavity (60)*	17.3 (7.9)	0.0730
	Oropharynx (240)	21.5 (6.6)			*Oropharynx (80)*	20.1 (7.6)	
Caries/Restoration	*Larynx (157)*	7.4 (4.6)			*Larynx (35)*	8.7 (5.8)	
	Oral Cavity (94)	7.6 (5.6)	0.0018*†		*Oral Cavity (60)*	6.8 (5.1)	0.0646
	Oropharynx (240)	9.1 (5.1)			*Oropharynx (80)*	8.8 (5.1)	
DMFT	*Larynx (157)*	18.2 (6.3)			*Larynx (35)*	18.8 (6.4)	
	Oral Cavity (94)	16.5 (7.8)	0.0004*†		*Oral Cavity (60)*	17.2 (7.2)	0.2623
	Oropharynx (240)	15.4 (6.7)			*Oropharynx (80)*	16.5 (6.9)	
Horizontal Bone Loss	*Larynx (157)*	8.9 (3.6)			*Larynx (35)*	7.5 (3.8)	
	Oral Cavity (94)	7.4 (4.0)	0.0000*†		*Oral Cavity (60)*	7.3 (3.5)	0.9002
	Oropharynx (240)	7.0 (3.6)			*Oropharynx (80)*	7.6 (4.1)	
Third Molars Present	*Larynx (157)*	1.0 (1.2)			*Larynx (35)*	1.2 (1.4)	
	Oral Cavity (94)	1.4 (1.4)	0.0020*†		*Oral Cavity (60)*	1.1 (1.2)	0.9245
	Oropharynx (240)	1.5 (1.5)			*Oropharynx (80)*	1.1 (1.4)	

Figure 3.10: Mean values for each dental domain with standard deviation in brackets based on gender. *One-way ANOVA with 95% confidence intervals with p values with those <0.05 marked by an *. Bonferroni correction (p<0.005) denoted as*

Larynx

Age range	25-34	35-44	45-54	55-64	65-74	75-84	85+	p value
N =	0	11	30	62	55	32	2	-
Teeth Present	-	18.4 (7.1)	21.2 (5.5)	17.2 (7.9)	16.0 (7.3)	15.4 (7.3)	17.0 (5.0)	0.0272*
Caries/Restored	-	7.9 (4.3)	7.6 (4.1)	7.8 (5.3)	7.9 (5.1)	6.8 (4.2)	7.0 (1.0)	0.9381
DMFT	-	17.6 (8.0)	14.2 (7.2)	18.5 (5.8)	19.9 (5.3)	19.1 (5.8)	18.0 (4.0)	0.0032*†
Horizontal Bone Loss	-	8.5 (2.5)	8.9 (3.8)	9.1 (3.7)	8.4 (4.0)	8.0 (3.4)	10.0 (3.0)	0.7648
Third molars present	-	1.4 (1.7)	1.6 (1.5)	1.0 (1.2)	1.0 (1.2)	0.7 (0.8)	2.0 (1.0)	0.0602
Oral Cavity								
N =	6	7	39	42	45	13	2	
Teeth Present	27.0 (1.4)	25.1 (2.9)	19.8 (6.3)	17.0 (7.5)	17.1 (8.6)	15.5 (6.9)	15.0 (1.0)	0.0024*†
Caries/Restored	3.7 (3.9)	6.1 (5.4)	7.2 (6.0)	7.2 (4.9)	8.0 (6.0)	7.1 (3.2)	9.5 (0.5)	0.6610
DMFT	4.7 (3.7)	9.1 (7.7)	15.5 (7.5)	17.9 (7.2)	18.8 (6.6)	18.9 (4.8)	21.5 (2.5)	0.0000*†
Horizontal Bone Loss	3.0 (3.0)	7.4 (3.6)	7.9 (3.8)	8.2 (3.6)	6.5 (3.9)	7.8 (2.4)	10.0 (0.0)	0.0207*
Third molars present	1.7 (1.5)	1.0 (1.3)	1.5 (1.4)	1.3 (1.3)	1.2 (1.3)	1.4 (1.5)	0.0 (0.0)	0.6897
Oropharynx								
N =	2	17	97	123	66	15		
Teeth Present	22.5 (5.5)	25.4 (3.0)	23.4 (5.6)	21.6 (6.1)	17.2 (8.0)	14.8 (6.5)	-	0.0000*†
Caries/Restored	3.5 (3.5)	6.5 (3.8)	8.3 (4.5)	10.1 (5.0)	8.8 (6.1)	9.0 (4.4)	-	0.0142*
DMFT	8.0 (8.0)	9.1 (5.4)	12.7 (6.5)	16.4 (5.7)	19.3 (5.8)	22.0 (4.1)	-	0.0000*†
Horizontal Bone Loss	1.5 (1.5)	5.7 (2.4)	6.2 (3.8)	7.6 (3.8)	8.0 (3.5)	8.2 (3.0)	-	0.0011*†
Third molars present	1.0 (0.0)	1.8 (1.5)	1.6 (1.6)	1.4 (1.5)	1.1 (1.3)	0.9 (0.9)	-	0.1774

Figure 3.11: Mean values for each dental domain with standard deviation in brackets based on age decades. *One-way ANOVA with 95% confidence intervals with p values with those <0.05 marked by an *. Bonferroni correction (p<0.003) denoted as †*

	Sub-site (N)	Smoker	p value	Sub-site (N)	Ex-smoker	p value	Sub-site (N)	Never smoked	p value
Teeth	*Larynx (104)*	16.4 (7.6)		*Larynx (76)*	17.3 (7.3)		*Larynx (12)*	23.8 (2.9)	
Present	*Oral Cavity (49)*	16.2 (8.0)		*Oral Cavity (55)*	19.1 (7.0)		*Oral Cavity (50)*	19.7 (7.7)	
	Oropharynx (106)	19.7 (7.5)	0.0026*†	*Oropharynx (131)*	21.1 (7.1)	0.0011*†	*Oropharynx (83)*	22.9 (5.0)	0.0067*
Caries/	*Larynx (104)*	7.2 (4.9)		*Larynx (76)*	7.7 (4.6)		*Larynx (12)*	10.4 (4.6)	
Restoration	*Oral Cavity (49)*	7.3 (5.6)		*Oral Cavity (55)*	8.4 (5.4)		*Oral Cavity (50)*	6.1 (5.3)	
	Oropharynx (106)	9.0 (5.6)	0.0332*	*Oropharynx (131)*	8.8 (4.9)	0.3030	*Oropharynx (83)*	9.3 (4.8)	0.0007*†
DMFT	*Larynx (104)*	18.6 (6.5)		*Larynx (76)*	18.5 (6.1)		*Larynx (12)*	14.0 (5.1)	
	Oral Cavity (49)	18.9 (6.6)		*Oral Cavity (55)*	17.2 (7.4)		*Oral Cavity (50)*	14.1 (7.9)	
	Oropharynx (106)	17.1 (7.1)	0.1720	*Oropharynx (131)*	15.5 (6.3)	0.0054*	*Oropharynx (83)*	14.1(6.5)	0.9989
Horizontal	*Larynx (104)*	8.8 (3.8)		*Larynx (76)*	8.8 (3.4)		*Larynx (12)*	6.6 (3.7)	
Bone Loss	*Oral Cavity (49)*	8.1 (4.4)		*Oral Cavity (55)*	7.5 (3.6)		*Oral Cavity (50)*	6.6 (3.2)	
	Oropharynx (106)	8.7 (3.8)	0.5704	*Oropharynx (131)*	6.8 (3.5)	0.0005*†	*Oropharynx (83)*	5.8 (3.3)	0.3549
Third	*Larynx (104)*	0.9 (1.2)		*Larynx (76)*	1.3 (1.3)		*Larynx (12)*	1.2 (1.0)	
Molars	*Oral Cavity (49)*	1.4 (1.2)		*Oral Cavity (55)*	1.1 (1.3)		*Oral Cavity (50)*	1.4 (1.4)	
Present	*Oropharynx (106)*	1.2 (1.4)	0.0575	*Oropharynx (131)*	1.3 (1.5)	0.6439	*Oropharynx (83)*	1.7 (1.5)	0.3404

Figure 3.12: Mean values for each dental domain with standard deviation in brackets based on smoking status. *One-way ANOVA with 95% confidence intervals with p values with those <0.05 marked by an *. Bonferroni correction (p<0.003) denoted as †*

Sub-site	Stage I (40/11/6)	p value	Stage II (37/9/26)	p value	Stage III (54/25/27)	p value	Stage IV (61/109/261)	p value
Teeth Present								
Larynx	16.1 (7.1)		17.1 (7.5)		17.9 (7.9)		17.4 (7.1)	
Oral Cavity	16.1 (6.0)	0.1336	18.0 (6.4)	0.7835	16.1 (8.3)	0.0037*	19.1 (7.6)	0.0002*†
Oropharynx	22.0 (4.2)		18.5 (8.9)		22.8 (5.4)		21.2 (6.8)	
Caries/Restoration								
Larynx	8.1 (4.9)		8.1 (5.0)		6.8 (4.5)		7.8 (4.8)	
Oral Cavity	8.5 (5.9)	0.9376	9.4 (3.5)	0.5942	7.1 (6.2)	0.0154*	7.0 (5.3)	0.0064*
Oropharynx	8.8 (5.3)		9.3 (5.6)		10.3 (5.5)		8.8 (5.0)	
DMFT								
Larynx	20.0 (5.3)		18.9 (6.3)		16.7 (7.0)		18.2 (6.1)	
Oral Cavity	20.5 (5.5)	0.0701	19.4 (4.7)	0.9644	18.4 (6.9)	0.2854	15.8 (7.9)	0.0184*
Oropharynx	14.7 (5.3)		18.7 (7.8)		15.5 (5.3)		15.4 (6.7)	
Horizontal Bone Loss								
Larynx	8.3 (3.4)		8.4 (3.6)		8.7 (3.7)		9.1 (3.9)	
Oral Cavity	7.7 (3.4)	0.6384	9.1 (4.0)	0.7708	7.7 (3.4)	0.3700	7.1 (3.9)	0.0004*†
Oropharynx	7.0 (3.4)		8.0 (4.5)		7.7 (3.7)		7.0 (3.7)	
Third Molars Present								
Larynx	0.9 (1.1)		0.9 (1.2)		1.2 (1.3)		1.1 (1.3)	
Oral Cavity	1.3 (0.9)	0.4660	1.0 (1.2)	0.5862	1.2 (1.2)	0.9449	1.4 (1.4)	0.3262
Oropharynx	1.3 (1.5)		1.2 (1.0)		1.3 (1.5)		1.4 (1.5)	

Figure 3.13: Mean values for each dental domain with standard deviation in brackets based on TNM7 staging. *One-way ANOVA with 95% confidence intervals with p values with those <0.05 marked by an *. Bonferroni correction (p<0.0025) denoted as †*

Sub-site	Stage I (40/11/26)	p value	Stage II (37/9/150)	p value	Stage III (54/25/68)	p value	Stage IV (61/109/72)	p value
Teeth Present								
Larynx	16.1 (7.1)		17.1 (7.5)		17.9 (7.9)		17.4 (7.1)	
Oral Cavity	16.1 (6.0)	0.0011*†	18.0 (6.4)	0.0005*†	16.1 (8.3)	0.0001*†	19.1 (7.6)	0.3530
Oropharynx	22.4 (6.5)		21.7 (6.4)		22.3 (5.8)		18.8 (7.7)	
Caries/Restoration								
Larynx	8.1 (4.9)		8.1 (5.0)		6.8 (4.5)		7.8 (4.8)	
Oral Cavity	8.5 (5.9)	0.1659	9.4 (3.5)	0.2709	7.1 (6.2)	0.0826	7.0 (5.3)	0.5726
Oropharynx	10.7 (6.1)		9.5 (4.7)		8.8 (5.2)		7.6 (5.3)	
DMFT								
Larynx	20.0 (5.3)		18.9 (6.3)		16.7 (7.0)		18.2 (6.1)	
Oral Cavity	20.5 (5.5)	0.0322*	19.4 (4.7)	0.0069*	18.4 (6.9)	0.0175*	15.8 (7.9)	0.1213
Oropharynx	16.3 (6.9)		15.6 (6.4)		14.3 (6.2)		16.7 (7.2)	
Horizontal Bone Loss								
Larynx	8.3 (3.4)		8.4 (3.6)		8.7 (3.7)		9.1 (3.9)	
Oral Cavity	7.7 (3.4)	0.0678	9.1 (4.0)	0.0060*	7.7 (3.4)	0.2136	7.1 (3.9)	0.0022*†
Oropharynx	6.2 (3.8)		6.5 (3.8)		7.6 (3.5)		8.5 (3.5)	
Third Molars Present								
Larynx	0.9 (1.1)		0.9 (1.2)		1.2 (1.3)		1.1 (1.3)	
Oral Cavity	1.3 (0.9)	0.2006	1.0 (1.2)	0.1107	1.2 (1.2)	0.2441	1.4 (1.4)	0.4119
Oropharynx	1.4 (1.3)		1.4 (1.4)		1.6 (1.6)		1.3 (1.5)	

Figure 3.14: Mean values for each dental domain with standard deviation in brackets based on TNM8 staging. *One-way ANOVA with 95% confidence intervals with p values with those <0.05 marked by an *. Bonferroni correction (p<0.0025) denoted as †.* Note: OPC totals 316 rather than 320 as 4 patients were excluded with unknown HPV status.

Oral Cavity	**Jaws (inc retromolar) (48)**	**Buccal mucosa and lip (19)**	**FOM (27)**	**Palate (8)**	**Tongue (51)**	**p value**
Teeth Present	17.9 (7.1)	18.5 (5.9)	16.3 (8.8)	14.8 (5.8)	20.4 (7.7)	0.1009
Caries/Restoration	6.7 (4.9)	8.0 (5.6)	6.4 (4.5)	8.4 (5.4)	7.9 (6.2)	0.6268
DMFT	16.5 (7.7)	17.5 (7.6)	18.0 (7.2)	21.4 (4.4)	15.3 (7.6)	0.2002
Horizontal Bone Loss	7.7 (3.7)	10.6 (6.4)	6.1 (3.7)	9.3 (2.8)	7.0 (3.9)	0.0044*
Third Molars Present	1.4 (1.4)	1.3 (1.3)	1.3 (1.3)	1.0 (1.0)	1.3 (1.4)	0.9589
Larynx	**Supraglottic (72)**	**Glottic (90)**	**Subglottic (30)**	**p value**		
Teeth Present	17.5 (7.2)	17.1 (7.5)	16.9 (7.9)	0.9138		
Caries/Restoration	7.6 (4.7)	8.1 (4.9)	6.2 (4.7)	0.1737		
DMFT	18.0 (6.5)	18.8 (6.0)	17.4 (6.7)	0.5129		
Horizontal Bone Loss	8.4 (3.9)	8.8 (3.2)	8.9 (4.4)	0.7344		
Third Molars Present	1.3 (1.4)	0.9 (1.1)	0.8 (1.1)	0.0633		
Oropharynx	**Tonsil (166)**	**BOT (130)**	**OPC (Other) (24)**	**p value**		
Teeth Present	21.9 (6.7)	20.2 (7.0)	20.6 (7.1)	0.1004		
Caries/Restoration	8.9 (5.3)	9.1 (4.9)	9.4 (5.4)	0.8815		
DMFT	14.9 (7.1)	16.6 (6.1)	16.7 (6.2)	0.0712		
Horizontal Bone Loss	6.9 (4.0)	7.5 (3.5)	6.9 (3.1)	0.3721		
Third Molars Present	1.5 (1.5)	1.2 (1.4)	1.7 (1.6)	0.1254		

Figure 3.15: Mean values for each dental domain with standard deviation in brackets based on micro-sites for each tumour sub-site. *One-way ANOVA with 95% confidence intervals with p values with those <0.05 marked by an *. Bonferroni correction (p<0.0022) denoted as †.*

3.5 Discussion

The current study assessed the dental status in various formats of newly diagnosed HNC patients due to commence IMRT. To understand the implications of their dental status it is imperative to compare them to their demographic equivalents in the general population. The current Adult Dental Health Survey (2009) provides reliable oversight of dental status for the UK (ADHS, 2009). Following this, sub-site comparison has been made to understand further implications within the HNC cohort. The current study provides the most recent data on both a changing landscape in dental health and HNC on the background of a novel RT delivery system (IMRT). This study is the largest HNC cohort study within a new dental and oncological era. Based on the latter this study considers variation in dental status based on HNC sub-sites.

3.5.1 Overall dental status of head and neck cancer patients

The retention of 21 or more natural teeth is widely used to define the minimum number of teeth consistent with a functional dentition (ADHS, 2009). In the current study, the mean number of teeth in both sexes fell short of this threshold at the time of their pre-RT dental assessment. In England, the mean number of teeth in the general population was 25.7 (ADHS, 2009) while in HNC patients this is 19.8, which suggests they continue to have sub-optimal dental health. This trend was one also alluded to by another UK based study (Critchlow et al., 2014), showing only 64% of HNC patients were able to meet the functional 21 teeth threshold. The direct clinical relevance of this is that further dental extractions are likely to have a deleterious impact on oral function. This would be compounded further with additional known post-RT effects such as trismus, xerostomia and dysphagia. Hence this presents a significant challenge to the dental oncologist when deciding upon the need for dental extractions.

It has become apparent that not all HNC sub-sites are impacted by tobacco and alcohol consumption. An obvious example is the role of viral infection such as Epstein-Barr virus in NPC and HPV in OPC. It is estimated that HPV induced tumours may account for 70-80% of OPCs in North America and parts of Europe (Boscolo-Rizzo et al., 2013; Erich M Sturgis & Ang, 2011; Zaravinos, 2014). The UK has seen a doubling in incidence of this disease between 1990-2006 and again between 2006-2010 (Mehanna. et al., 2016). This changing landscape means OPC is now responsible for almost half of all HNCs. Hence, a

substantial proportion of current HNC patients have a completely different dental profile to the traditional patient. Importantly, HPV infection has no direct impact on dental disease (Tezal et al., 2013). Consequently, a blanket dental protocol for HNC patients is impractical and wrong. Dental assessment and treatment planning should be considered at the sub-site level and preferably per individual patient a theme previously highlighted by Maier et al some 20 years ago (Maier et al., 1993). OCC, LC and OPC are the most common tumour sub-sites accounting for over 80% of all HN SCC (DAHNO, 2014). It is important that the dental status of these tumour groups is better understood in order to better tailor management to patient needs.

3.5.2 Historical pre-RT dental studies

There is limited information regarding the dental status of HNC patients in the pre-RT phase of treatment. Most studies are historic and do not reflect the current dental status in the population. This is evident (Critchlow et al., 2014; Gaggenheimer & Hoffman, 1994) when comparing the study by Gaggenheimer et al. between 1965-1990 with Critchlow et al (2013). In the former population of 947 HNC patients, 59% were edentulous (Gaggenheimer & Hoffman, 1994), 14% had a poor dentition and 18% an intact dentition (Gaggenheimer & Hoffman, 1994) compared to the 2013 cohort where only 2% of HNC patients were edentulous (Critchlow et al., 2014). This changing pattern of tooth retention is one confirmed by ADHS (ADHS, 2009).

Another difference is the rate of dental caries. ADHS reported 31% of the general population to have dental decay (ADHS, 2009) in contrast to 71% (Lockhart & Clark, 1994) reported by Lockhart & Clark and 61% (Critchlow et al., 2014) reported by Critchlow et al in the HNC population. The present cohort had a mean of 8.2 carious/restored teeth and a minimum of 41% (8.2/19.8) were candidates for extraction either pre- or post-RT with the risk continuing over time. The mean DMFT in the study population was 16.2, which was similar to that of Rouers et al (DMFT 16.1) (Rouers et al., 2016) but less than that in earlier studies where values of 18.1 (Moraes et al., 2016) and 19.6 (Tezal et al., 2013) were reported. The high DMFT scores reflect the current and historical burden of dental disease in the population.

The third component is periodontal disease which is prevalent in all studies reporting on HNC patients ranging from 40% (Niewald et al., 2013) to 80% (Critchlow et al., 2014;

Moraes et al., 2016). The present study assessed HBL from radiographs and moderate (7.4) alveolar bone loss was evident. Lockhart & Clark reported bone loss of 66% in HNC patients (Lockhart & Clark, 1994) compared to 45% in the general population (ADHS, 2009) and the difference probably relates in part to smoking habits. The relevance of identifying periodontal disease is the increased risk of ORN (Schuurhuis et al., 2011). If poor or hopeless teeth are not extracted, then the incidence of ORN rises to 33% (Schuurhuis et al., 2011). The need for balance dental extractions in preparing the patient for treatment is obvious.

3.5.3 Gender

Data from the ADHS survey identifies men as having more natural teeth, dental decay and periodontal disease compared to females (ADHS, 2009). In the current cohort there were no significant differences in dental statuses by gender except for the presence of third molars. Males had 1.4 third molars compared to 1.1 in females. ORN, is more prevalent in men (Reuther et al., 2003) with the most favoured site being the mandibular third molar region. Hence, non-ectopic impacted third molars should be considered for extraction at the pre-RT phase. In 2000, the national institute of clinical excellence (NICE) published guidance for third molar surgery (NICE, 2000). The net result is that more wisdom teeth were being retained in the population. Our data shows that patients impacted by this guidance (age group 29-52Y) had the highest number of third molars. The concern regarding the retention of third molars is that they commonly succumb to dental disease (McArdle & Renton, 2012) over time and their position in the jaws coincides with radiation hot spots and which leads to vulnerability to ORN.

3.5.3.1 Gender and dental status in common HNCs

Comparing genders independently for dental differences for common tumour sub-sites (Figure 3.10) identified in all 5 dental categories, OPC males to have both a better and more complex dentition compared to LC and OCC. Essentially, the exact opposite was true for male LC patients whom have likely lost many teeth via periodontal disease secondary to habitual and long-standing smoking.

In the current cohort, over a third (n = 240/666, 36%) of the group consisted of male OPC patients. This identifies this specific cohort as both a populous and vulnerable

demographic group. One concern is their higher retention of third molars, a favoured ORN site. With the addition of a vastly superior dentition constantly under threat post-RT it appears no coincidence that this populated group suffers the most from ORN.

3.5.4 Age

The number of teeth is strongly associated with age, reflecting the one-way nature of tooth loss and the accumulated effects of dental disease and treatment over the life course (ADHS, 2009). It is therefore not surprising that all the aforementioned dental parameters deteriorate with increasing age. Today, adults <65 years of age rarely lose all their natural teeth (ADHS, 2009) but those >45 years still carry the legacy of dental decay and heavily restored teeth which means fewer sound teeth in the mouth (ADHS, 2009). The reality is that post-RT these teeth are vulnerable to dental disease and with it the risk of ORN. In this study, age appeared to be an important factor when considering the 21 natural teeth threshold for a functional dentition. A pivotal point of change was identified between age decade 45-54 and 55-64. Between these decades the mean number of teeth present shifted from 22.3 to 19.7 respectively. This finding is even more potent as the decade 55-64 was the peak age range for cancer diagnosis accounting for a third of the whole cohort.

With increasing age (≥45-54) there is an emergence of root caries (ADHS, 2009). This specific dental disease is particularly accentuated by xerostomia in the post RT phase. In the present study 35-53% of teeth in patients age 45-90 were either decayed or vulnerable to secondary decay at the pre-RT phase. What is clear is that in the 21st century patients aged 45-90 (82% of the cohort) present with complex dental issues that pose a real dilemma as to how to achieve optimum post-RT function and QoL from an oral and dental status.

3.5.4.1 Age and dental status in common HNCs

The incidence of OPC, LC and OCC essentially peaked at 55-64 year of age. Differences in dental status were clearly evident within this decade and hence cannot purely be denoted to age-related dental disease. Increasing age identified worsening HBL scores in both OCC and OPC but not in LC. In contrast, the latter consistently showed sustained poor HBL scores over decades and this undoubtedly is due to their heavy and long-standing smoking habits. The importance of periodontal disease is at times understated but is a

genuine risk factor for ORN. HNC patients with periodontitis are at an increased risk (19-33%) for ORN (Schuurhuis et al., 2011).

The natural progression of tooth loss in each sub-site is an interesting finding and one that may provide another factor towards the rising rate of ORN in OPC patients. From the peak decade of 55-64Y to 65-74Y, OPC patients lose 4 teeth whereas OCC and LC patients on average only lose 1 tooth. Therefore, if OPC patients were destined to lose teeth regardless of their cancer, now with the background of RT it will only drive the ORN rate up in this group who have a high survivorship. Yet again data suggests that this is because the OPC group have a more complex retained dentition compared to LC and OCC. At this peak cancer age, almost 50% of the dentition in OPC patients was either carious or restored highlighting the high dental need this group requires.

3.5.5 Smoking status

Smoking has a significant role in HNC and in the current cohort 72% of patients were either smokers or ex-smokers. Smoking has strong links to oral health with dentate adults who had never smoked more likely to have excellent oral health compared to current or ex-smokers (ADHS, 2009). In the study cohort smoking status was significantly related to number of teeth present, DMFT score, HBL and third molars being present. Furthermore, smoking increases xerostomia, which invites caries (Rad et al., 2010).

3.5.5.1 Smoking status and dental status in common HNCs

In active smokers, OPC patients had almost 4 teeth more that either OCC or LC patients. Considering that peak ages of all 3 sub-sites are essentially the same tooth loss cannot be related to age only. This trend was the same in ex-smokers too. A possible explanation may be that OPC patients prioritise dental health and therefore retain teeth for longer. Alternatively, generalised smoking habits may also influence this outcome. LC and OCC patients may have lost their teeth sooner from periodontal disease based on higher and longer tobacco use. In contrast, OPC smokers may have a more casual or social habit and therefore less impactful on their teeth. The current study did not collect pack years so no definitive reason can be stated. Patients who reportedly never smoked identified LC patients on average as having the most teeth present. This further implicates the role of smoking and tooth loss in this cohort of patient.

3.5.6 Tumour size

For both caries/restorations and HBL, tumour size was statistically significant with the trends suggesting that larger tumour sizes are related to irregular dental attendance. Therefore, pre-RT preparation is likely to result in extraction rather restoration due to unrestorable teeth or periodontal disease.

3.5.7 Nodal size

Enlarging nodal size identified an increase in both mean number of teeth and third molar presence. This finding is likely linked to the increasing number of OPC patients which accounted for 36% (320/886) of the cohort. OPC patients commonly present with painless neck lumps (Marur & Forastiere, 2008; O'Sullivan et al., 2012). Ang et al reported that over 66% (478/721) of patients presented with advanced classification (N2 or N3) nodal disease (Ang et al., 2010). Many of these patients do not have the conventional HNC risk factors of tobacco exposure and excessive alcohol use but rather related to HPV. Hence, the lack of traditional risk factors means the dentition does not reflect the 'stereotyped' HNC dentition and therefore the retention of more teeth. The immediate concern is a higher nodal staging encompasses a wider area being irradiated involving a larger proportion of the dentition. This coupled with high survivorship in HPV positive OPC (Ang et al., 2010) could explain why this sub-group of patients are at a heightened risk of ORN. The presence of more third molars in higher nodal size therefore requires meticulous assessment at the pre-RT phase. The radiation dose to this region in higher nodal stages is likely to exceed the 40Gy threshold in developing ORN.

3.5.8 Staging

3.5.8.1 Staging via TNM7

Staging the 3 common sub-sites by TNM7 identified that OPC patients presented with a better dentition and not dentally equivalent to LC and OCC patients. Clearly OPC patients have a differing dental profile possibly from prioritising dental health, retaining and restoring teeth and differing smoking and alcohol habits. One pertinent finding in staging

via TNM7 was that OPC group had more than double the number of OCC patients and more than quadruple the number of LC patients in stage IV. This finding should not be overlooked. Protocol dictates that TNM7 stage IV OPC patients receive combined chemo-RT. RT would routinely involve both necks and will involve a large span of the teeth and jaws. This could yet be another factor possibly contributing to the rise of ORN in this group.

3.5.8.2 Staging via TNM8

More recently, a revised staging system (TNM8) has been proposed. The major change in TNM8 is splitting OPC by their HPV status. HPV positive have been de-escalated in their staging whereas HPV negative and LC have stayed the same as TNM7. Interestingly, re-staging this cohort found OPC patients now have better dentitions in the lower stages mimicking the de-escalating pattern of HPV positive patients. This pattern alone strengthens the concept that within OPC, particularly the HPV positive patients have a superior dentition. In contrast, all 3 sub-sites in TNM8 stage IV had equivalent dentitions suggesting HPV negative, LC and OCC share large similarities. All 3 of these tumour sub-sites are heavily linked to smoking and alcohol consumption and these habits would have left a telling mark on the dentition. Their dental behaviours are also likely to be similar with sporadic dental attendance and neglected oral hygiene.

3.5.9 Sub-sites

Differential appreciation of HNC sub-site is an essential factor during pre-RT dental assessment. Distinct differences in RT coverage for various sub-sites are recognised and should be considered during treatment planning. In tooth bearing areas, LC receive less than 25Gy (Bak et al., 2016). In contrast, OPCs and hypopharyngeal cancers receive in excess of 50Gy (Bak et al., 2016). In NPCs, maxillary teeth have a higher dose of RT than the mandibular teeth (Bak et al., 2016). However, though various groups have been deemed vulnerable to ORN it is their dental status which will compound their true risk. The long-term presence of teeth, survivorship and late effects of radiation induced fibrosis will lead to ORN. It is on this principle patients with OPC and NPC have such an elevated risk. The clear difference in dental status based on sub-site is most evident with the strong significance found in this study. However, dividing sub-sites further by the micro-site did not appear to be of any clinical significance for dental status.

Tumour sites heavily linked with excessive alcohol and tobacco exposure had on average < 20 teeth on presentation, whereas those sub-sites less heavily linked to these risk factors had >20 teeth on average. Compared to Maier et al who reported on pre-RT caries via sub-site, our study showed almost identical figures of carious and restored teeth for LC, OCC and hypopharyngeal cancer (Maier et al., 1993). However, in contrast this study showed OPC patients had a higher number of carious/restored teeth with the likelihood the number reflects more repaired teeth which is vastly different to that reported by Maier et al (Maier et al., 1993). This finding would suggest a lack of dental behavioral change regarding oral health of tobacco and alcohol related HNC sub-sites. Hence, OPC, over the last 2 decades has shifted from traditional factors to a HPV driven cause and this is reflected in a better dentition.

One very interesting finding in this study was the dental status of the 'unknown HNC primary'. Patients who had attended with a neck lump with a positive diagnosis of squamous cell carcinoma and no obvious solid tumour identified were diagnosed under this category. More recently, it has been determined that these patients are likely to have micro-tumours in the oropharynx which cannot be visualized. Hence mucosectomy via robotic surgery have been proposed. In the current cohort 49 patients were deemed to have an unknown HNC primary. Interestingly when assessing their dental status and comparing this to the OPC group of 320 patients the mean of each category is almost identical with the standard deviation differences less than 1 tooth apart. This dental trend of such similarity essentially provides a blind test, which fits the narrative, that the unknown HNC primary group were most likely OPC patients.

The current population study reviewed all patients attending for a pre-RT dental assessment on a continuous basis from 2011-2017. Amongst this population OPC was the most populated group with over a third of all cases. If the unknown HNC primary group is coalesced with OPC this equates to 42% of all cases. With the phenomenal rise of OPC with high survivorship (Mehanna. et al., 2016) dental oncologists need to urgently re-evaluate dental management approach in this group. The balance of maintaining a functional dentition while avoiding ORN in a time critical assessment period is a significant challenge. Equally, oncologist need to recognize the challenges faced by dental oncologist in sub-groups with oral health landscape also changing.

3.6 Conclusion

There are distinct differences in the dental status of HNC patient due to commence RT compared to the general population. Within HNC, dental status varies based on cancer sub-site and this should be considered during dental assessment to tailor a dental treatment plan. Consideration should be given to balancing function versus avoidance of ORN on the background of survivorship.

Amongst the 3 most common HNCs, OPC patients have a better dentition compared to LC and OCC. The significance of an improved dentition on the background of post-RT survivorship provides an insight towards a rise of ORN in this tumour group. Prophylactic molar extractions to restrict ORN pre-RT remain a controversial practice. Ironically, pre-RT dental extractions have been highlighted for causing ORN itself (N. M. Beech et al., 2017; Chang et al., 2007). Such invasive treatment coupled with expedited RT pathways in OPC (Grønhøj et al., 2018) exposes the most vulnerable and unhealed extraction sites to high levels of radiation. It is a practice that requires re-evaluating and particularly in male OPC patients. Such practice will immediately reduce this group to <21 teeth, below the threshold of a functional (ADHS, 2009) dentition. Oral function will only worsen following post-RT trismus, dysphagia and xerostomia. With high survivorship (Ang et al., 2010), a concerted effort towards tooth retention and dental health should be considered in this group.

A clear distinction exists between the dental status within the OPC cancer group in relation to HPV status. Survivorship also differs with HPV positive patients having vastly better outcomes (Ang et al., 2010). Therefore, OPC should be assessed based on HPV differences with more detail to determine whether it is a substantial factor.

3.7 Limitations

Though this study includes a large cohort of patients a number of limitations have been recognised. The study used radiographs alone to determine dental status without incorporating clinical examination data. Two main limitations have been identified. Firstly, radiographic HBL reflects historic periodontal disease and does not inform on

current disease status. Secondly, third molars were recorded as present or absent on radiographs, as their eruptive status based on imaging only cannot be determined

Author	Tumour group (total number)	Key Findings
(Maier et al., 1993)	HNC (100)	Decayed teeth: 24.3% laryngeal cancer, 28.9% oral cancer, 29.6% in the hypopharynx and 48.7% in oropharynx
(Gaggenheimer & Hoffman, 1994)	OCC & OPC (947)	Dental status of patients between 1965-1990 Edentulous: 59% Intact dentition: 18%
(Lockhart & Clark, 1994)	HNC (131)	Alveolar bone loss 66%, clinical caries 71%, failing restorations 91%
(Jham et al., 2008)	HNC (207)	Periodontal disease 41%, residual root 21.2%, caries 12%
(Frydrych & Slack - Smith, 2011)	OCC & OPC (127)	No association was found between dental attendance and gender, smoking, disease stage or age at diagnosis.
(Niewald et al., 2013)	OCC (90)	Patients' dental status before radiotherapy was generally poor. 10 teeth were present; six of them were regarded to remain conservable. 40% had chronic periodontitis
(Critchlow et al., 2014)	HNC (100)	2% were edentulous Dentate patients, 71% had periodontal disease The mean number of carious teeth per dentate subject was 2.4 61% of dentate patients presented with one or more carious teeth 64% (64/100) had 21 or more natural teeth. The mean DMFT score was 19.6
(Tezal et al., 2013)	HNC (399)	Lower mean number of teeth with caries, crowns, endodontic treatments and fillings
(Rouers et al., 2016)	HNC (48)	Mean DMFT 16.1
(Moraes et al., 2016)	OCC & OPC (35)	80% of patients had chronic generalized periodontitis Median DMFT was 18.1

Figure 3.16: Presents a summary of studies within the literature regarding the dental status of head and neck cancer patients upon diagnosis

Chapter 4

The dental status of oropharyngeal cancer patients specifically prior to commencing intensity modulated radiation treatment

4.1 Introduction

The previous chapter explored dental status at diagnosis of HNC patients in the IMRT era (Vinod. Patel et al., 2020; V. Patel et al., 2020). The study identified variation in dental status by tumour sub-site, which should be taken into consideration when planning dental intervention. Furthermore, comparing the 3 most common HNC sites identified OPC patients having both a better and a more complex dentition to manage (Vinod. Patel et al., 2020). The significance of this observation is that the pattern of HNC is changing with an upsurge in the incidence of OPC (Ernster et al., 2007; A. Jemal et al., 2008; E. M. Sturgis & Cinciripini, 2007). This rise has been linked to HPV and is seen more in a younger population (A. Jemal et al., 2008) than the typical HNC patient. OPC responds well to RT and data shows that outcomes are improved by early initiation of RT resulting in commencement of treatment within 14 days (Grønhøj et al., 2018). This places inordinate demands on a dental oncology service.

Over the past 3 decades a phenomenal rise in OPC has been reported (de Martel et al., 2012). Based on this trend, OPC in England is estimated to increase by 239% between 2011-2025 accounting for 35% of all HNC (Chaturvedi et al., 2011; Louie et al., 2015). Simultaneously, while HPV positive OPC continues to increase HPV negative OPC tumours have significantly decreased (de Martel et al., 2012). The relevance of these facts is that HPV-positive OPC is seen mostly in middle-aged white men (40 to 59 years old) (Chaturvedi et al., 2008) and prognosis of HPV positive patients is favourable even with advanced disease (Ang et al., 2010; Li et al., 2003; O'Sullivan et al., 2012; Ragin & Taioli, 2007). This places an onus on the dental oncologist to tailor treatment to this emerging group such that they achieve optimal dental function over their life span. Liberal dental extractions are no longer the solution.

HPV positive and HPV negative tumours constitute two different disease entities with different biological and clinical characteristics (Ndiaye et al., 2014). Hence, determining HPV status provides invaluable knowledge when considering tumour management. It is widely reported (Ang et al., 2010; Ang & Sturgis, 2012; Li et al., 2003; O'Sullivan et al., 2012; Pytynia et al., 2014; Ragin & Taioli, 2007) that HPV positive OPC have significantly improved overall- and disease-free survival outcomes compared to site- and stage-matched HPV negative tumours (Pytynia et al., 2014). However, not all HPV positive OPC have favourable outcomes with a significant minority experiencing treatment resistance

resulting in poor outcomes (Masterson et al., 2014; Mirghani & Blanchard, 2018). Two factors associated with a negative outcome are heavy smokers (>10 pack years smoking)(Adelstein et al., 2009; Ang et al., 2010; Gillison, Zhang, et al., 2012; Sethi et al., 2012) and advanced nodal disease (N2b disease or above) (Ang et al., 2010). Though these factors have been validated (Ang et al., 2010; Granata et al., 2012) there is still no widely accepted strategy for identifying high-risk HPV positive patients. Equally, no definitive criteria to identify HPV associated OPC patients with poor outcome are available either (Ruangritchankul et al., 2019). Recently, several studies utilising lymphocyte subpopulation-specific detection techniques have demonstrated a positive correlation between tumour-infiltrating lymphocytes (TILs) and improved survival in HPV associated OPC (Balermpas et al., 2016; Nordfors et al., 2013; Oguejiofor et al., 2015; Solomon et al., 2018; Wansom et al., 2012; Ward et al., 2014). Using this method, a recent study (Ruangritchankul et al., 2019) grading TILs into high and low density showed the former had a significantly better overall and disease-free survival. Having a view on long-term survival is a key consideration when providing a pre-RT dental assessment. The impact of pre-RT dental extraction on QoL (N. Beech et al., 2016) and emotional status of the process has predominantly been negative (Clough et al., 2018). With more OPC patients surviving their disease the dental oncologist must consider a more personalised dental treatment plan. Consequently, it is important to identify the factors that impact the dental status within the cancer journey. Factors such as gender, age, smoking status, anatomical site and cancer staging are common demographics routinely used to judge survival. Equally many of these factors are also important in dental status.

Hence, the primary objective of this study was to now focus exclusively on OPC patients and explore these factors and their impact on the presenting dental status. There is little known about the dental status of OPC based on HPV status. The secondary objective was to determine whether the HPV positive patients when divided further based on TILs showed to have a difference in their presenting dentition. To the best of our knowledge a study to investigate and report on the pre-RT dentition of HPV positive patients based on their TILs status in the IMRT era has not been assessed.

4.2 Methodology

4.2.1 Ethics

19/EE/0224 - Dental status, radiotherapy doses and subsequent implications in head and neck cancer patients - A retrospective cohort study

4.2.2 Dental data collection

The current study is an extension of that described in chapter 3. Dental data collection was identical to that described in section 3.2.2. Specifically, for OPC additional information regarding HPV status was also collected.

4.2.3 TILs

4.2.3.1 TILs grading

TILs grading was completed in a previous study by Ruangritchankul et al as per the description in their publication (Ruangritchankul et al., 2019). HPV testing was undertaken at the time of diagnosis according to current guidelines (NICE, 2018). p16 immunohistochemistry (clone E6H4, CINtec, Roche, UK) was performed on an automated platform (Benchmark Ultra, Ventana Medical Systems, USA) according to manufacturer's instruction as previously described (S. Thavaraj et al., 2011). OPC demonstrating strong and diffuse nuclear and cytoplasmic positivity in >70% of tumour cells were then subject to high-risk HPV testing by DNA in-situ hybridisation (INFORM Family III, Roche, UK) according to manufacturer's instruction as previously described (S. Thavaraj et al., 2011). Only OPC demonstrating positivity for both p16 immunohistochemistry and high-risk HPV DNA by in-situ hybridisation were included in this study. TILs were evaluated on at least one representative whole-mount diagnostic haemotoxylin and eosin slide from the primary tumour (Ruangritchankul et al., 2019). Two consultant pathologists independently scored TILs according to a binary classification system as described by Ward and colleagues (Ward et al., 2014). Lymphocytes present within tumour nests/sheets, in the stromal component between tumour nests and in the normal lymphoid component of the tonsil and base of tongue were included. Any lymphocytes beyond the tumour invasive front, plasma cells and neutrophils were excluded from TILs assessment (Ruangritchankul et al., 2019). Whole-section area was then categorised as high (TILs-high; diffuse or patchy, present in >20% of tumour and stroma) or low (TILs-

low; sparse or absent, present in <20% of tumour and stroma) (Ruangritchankul et al., 2019).

4.2.3.2 TILs data

Data from the dental status cohort of HPV positive patients only was cross referenced with the existing TILs database compiled by Ruangritchankul et al (Ruangritchankul et al., 2019).

4.3 Statistical analysis

4.3.1 Dental status analysis

The sample and outcome data were summarised using descriptive statistics. The mean dental status based on gender, smoking, anatomical site, TNM staging was compared using unpaired t-test. The dental status for age was compared using one-way ANOVA. Significance was assumed at the 5% level, and analyses were done with the help of IBM SPSS Statistics for Windows, version 23.0 (IBM Corp).

4.3.2 TILs analysis

TILS data underwent inter-rater agreement analyses, which were calculated using SPSS for Windows version 25.0 (IBM, Portsmouth, UK). Kappa and weighted kappa statistics was used to evaluate inter-rater reliability (Ruangritchankul et al., 2019).

4.3.3 Dental status and TILs analysis in the HPV positive sub-group

TILs data was patient matched to dental data and subsequently summarised using descriptive statistics. The mean dental status based on gender, age, smoking, anatomical site and TNM staging was compared based on TILs-high and TILs-low sub-divisions. Analysis was undertaken using unpaired t-test and one-way ANOVA. Significance was assumed at the 5% level, and analyses were done with the help of IBM SPSS Statistics for Windows, version 23.0 (IBM Corp).

4.4 Results

4.4.1 Dental status of oropharyngeal cancer

A total of 320 OPC patients were identified with 316 patients with known HPV status (Figure 4.1). Males dominated (n=239, 75.6%) with the majority being HPV positive (n=172, 72%). The peak decade was 55-64 years and accounted for 38.3% (n=121) of the overall cohort. The majority of patients were ex-smokers (n= 130, 41.1%) and tonsillar tumours (n=166, 52.5%) were the most populated sub-site. Based on TNM7, stage IV was the largest group (n=258, 81.6%), however, following re-staging in accordance with TNM8, stage II (n=150, 47.5%) became the most common.

A comparison of dental health between HPV positive and HPV negative cases showed (Figure 4.2), the positive group had more teeth present (p=0.0000), a higher number of carious/restored teeth (p=0.0277), lower DMFT (p=0.0202) and less HBL (p=0.0000) compared to the negative group. Overall, they had better dental health.

A comparison by gender ignoring HPV status (Figure 4.3) revealed no difference in dental status except males retained more third molars (1.5 vs 1.1, p=0.0366). When HPV status was added to the gender equation (Figure 4.4), HPV positive males had more teeth (22.7 vs 18.4, p<0.0001), more carious/restored teeth (9.7 vs 7.6, p<0.0044) and less HBL (6.4 vs 8.6, p=0.0001) compared to negative males. In females, the HPV positive group had a significantly lower HBL score than the negative group (6.5 vs 9.1, p=0.0039). When comparing the HPV positive groups (Figure 4.5), males had significantly more teeth (22.7 vs 20.5, p=0.034) than positive females.

Age and dental status had an association (Figure 4.6) with increasing age correlated with significantly less teeth, higher DMFT and increasing HBL scores for all OPC patients (p<0.001) a pattern that mirrored the general population. When patients were divided by their HPV status then increasing age was associated with less teeth and higher DMFT (p<0.001) in both HPV positive and negative groups. Only HPV positive patients showed increase HBL scores with age (p<0.001). Direct comparison of the dental status of HPV positive vs negative (Figure 4.7) demonstrated patients aged 55-64Y had significantly more teeth (p=0.0001) (23.3 vs 17.8) a better DMFT score (15.2 vs 19.2) and better HBL score (6.8 vs 9.7). HPV positive patients have better dentition and dental health at presentation.

Smoking status had a significant impact on teeth present, DMFT and HBL scores with those that have never smoked (NS) having a better dentition compared to current smokers (CS) and ex-smokers (ES) (Figure 4.8). Focusing on HPV positive patients only, HBL severity and smoking status was highly significant (p=0.0000) with CS again with the worst scores. In contrast, smoking status had no impact on dental status in the HPV negative group (Figure 4.9). Comparing opposing HPV statuses (Figure 4.10) identified that only ES had a significant difference in their teeth with the HPV positive group once again identified to have a superior presenting dentition.

The dental status was similar between tonsil, base of tongue (BOT) and OPC (other) (Figure 4.11). However, this changed if HPV status was taken into consideration (Figure 4.12). In the HPV positive group patients with BOT tumours had the least teeth present (20.1, p=0.0017) and highest DMFT score (16.6, p=0.0095). Direct comparison (Figure 4.13) of HPV positive versus negative (Figure 4.13) patients revealed tonsillar tumours had a better dentition based on more teeth present (p<0.0001), lower DMFT (p=0.0023) and less HBL (p<0.0001).

No significance was seen in the dental status when patients were staged in accordance to TNM7 (Figure 4.14). However, patients re-staged based on TNM8 (Figure 4.15) revealed teeth present (I 22.4, II 21.7, III 22.3, IV 18.8 p=0.005) and HBL scores (I 6.2, II 6.5, III 7.6, IV 8.5 p=0.0008) as significant. Figure 4.16 compared equivalent stages of TNM7 versus TNM8 with only HBL seen as significant in stage IV. The obvious difference being that the TMN8 group is populated with only HPV negative patients whom commonly have a smoking history and therefore subsequently seen to have higher periodontal disease. Within TNM7 (Figure 4.17), HBL was significant between HPV positive and negative patients at stage II (5.6 vs 10.2, p=0.0093), stage III (6.0 vs 9.5 p=0.0094) and stage IV (6.5 vs 8.5, p=0.0001). In addition, stage IV HPV positive patients had more teeth (22.2 vs 18.8, p=0.0002) and more caries/restoration (p=0.0092). Following re-staging to TNM8 and direct comparison of HPV positive to negative, only HBL (6.2 vs 10.2, p=0.0002) in stage II was found to be significant of all stages and dental status categories. Interestingly, direct comparison of TNM7 versus TNM8 stages for HPV positive patients showed no statistical significance in any dental criteria (Figure 4.18).

4.4.2 Dental status based on TILs in the HPV positive sub-group

The current study had a population of 157 HPV positive OPC patients. Sub-dividing patients by TILs identified TILs-high to the most popular grading (n=140, 89%). In all demographic categories and sub-categories TILs-high over-whelming dominated TILs-low. Figure 4.19 shows the population demographics and the split via TILs-high and TILs-low.

The dental status of patients based on their TILs classification (Figure 4.20) showed no statistical significance. Observationally, both groups had similar dentitions with TILs-low figures marginally worse.

Using gender (Figure 4.21), smoking status (Figure 4.23) and OPC sub-site (Figure 4.24) as differentiator no statistical significance was identified between TILs-high and TILs-low patients though minor observational difference existed.

Both DMFT (p=0.0072) and third molar presence (p=0.0177) were identified as significant dental categories based on age (Figure 4.22) when considering the whole population. However, direct comparison of TILs-high versus TILs-low for each decade did not identify any particular age group to be significant.

Patients were staged via both TNM7 and TNM8 (Figure 4.25). No statistical significance was seen in the former staging in any dental criteria when sub-divided by TILs scoring. However, in TNM8, DMFT (p=0.0445) for the overall population was significant. Furthermore, within stage III, TILs-high patients presented with more third molars than TILs-low (p=0.0307).

Attribute		Total	HPV Status	
			Positive	Negative
Gender	Male	239 (240)	172	67
	Female	77 (80)	43	34
Age	25-34	2 (2)	2	0
	35-44	17 (17)	11	6
	45-54	97 (97)	71	26
	55-64	121 (123)	84	37
	65-74	64 (66)	38	26
	75-84	15 (15)	9	6
Smoking	Current	104 (106)	49	55
	Ex-smoker	130 (131)	93	37
	Never	82 (83)	73	9
Sub-site	Tonsil	166 (166)	123	43
	BoT	127 (130)	82	45
	OP - other	23 (24)	10	13
TNM 7	I	6 (6)	3	3
	II	25 (26)	12	13
	III	27 (27)	14	13
	IV	258 (261)	186	72
TNM 8	I	26	23	3
	II	150	137	13
	III	68	55	13
	IV	72	0	72

Figure 4.1: A breakdown of the patient cohort by various demographics. Two figures are presented in all section apart from TNM8. Numbers in the brackets are the total when including patients with unknown HPV status.

Oropharyngeal Tumour	Teeth Present	Carious or restored teeth	DMFT	Horizontal bone loss	Third molar present
HPV Status					
Positive (215)	22.3 (6.0)	9.5 (4.9)	15.0 (6.4)	6.5 (3.6)	1.5 (1.5)
Negative (101)	19.0 (7.7)	8.1 (5.4)	17.0 (7.0)	8.8 (3.6)	1.2 (1.4)
Unknown (4)	12.5 (9.2)	5.5 (1.5)	20.0 (11.2)	5.0 (3.1)	0.8 (1.3)
P value	**0.0000*#**	**0.0277***	**0.0202***	**0.0000*#**	**0.1719**

Figure 4.2: Mean values with standard deviation in brackets for each dental domain based on human papilloma virus (HPV) status of established oropharynx cancer. The lower section assesses dental status *One-way ANOVA with 95% confidence intervals. p values with those <0.05 marked by an *. Bonferroni correction (p<0.01) denoted as #*

	Male (240)	Female (80)	p value
Teeth Present	21.5 (6.6)	20.1 (7.6)	**0.1150**
Caries/Restoration	9.1 (5.1)	8.8 (5.1)	**0.6490**
DMFT	15.4 (6.7)	16.5 (6.9)	**0.2078**
Horizontal Bone Loss	7.0 (3.6)	7.6 (4.1)	**0.2137**
Third Molars Present	1.5 (1.5)	1.1 (1.4)	**0.0366***

Figure 4.3: Overall dental status of 320 OPC patients based on gender. *Unpaired t test with 95% confidence interval. Two-tailed p value < 0.05 is denoted as * with Bonferroni correction denoted as #*

	HPV Status	Teeth Present	Carious or restored teeth	DMFT	Horizontal bone loss	Third molar present
Male	HPV +ve (172)	22.7 (5.6)	9.7 (4.9)	14.7 (6.2)	6.4 (3.5)	1.6 (1.5)
	HPV –ve (67)	18.4 (7.9)	7.6 (5.5)	17.1 (7.3)	8.6 (3.5)	1.3 (1.5)
P value		**<0.0001*#**	**0.0044*#**	**0.0113**	**0.0001*#**	**0.1662**
Female	HPV +ve (43)	20.5 (7.6)	8.8 (5.2)	16.3 (6.8)	6.5 (3.8)	1.1 (1.4)
	HPV –ve (34)	20.1 (7.2)	9.0 (5.1)	16.7 (6.3)	9.1 (3.8)	1.0 (1.3)
P value		**0.8151**	**0.8662**	**0.7920**	**0.0039*#**	**0.7490**

Figure 4.4: Compares the dental status of males and females based on their HPV status. Unpaired t test with 95% confidence interval. Two-tailed p value < 0.05 is denoted as *. *Bonferroni correction (p<0.005) denoted as #*

	Gender	Teeth Present	Carious or restored teeth	DMFT	Horizontal bone loss	Third molar present
HPV +ve	Male (172)	22.7 (5.6)	9.7 (4.9)	14.7 (6.2)	6.4 (3.5)	1.6 (1.5)
	Female (43)	20.5 (7.6)	8.8 (5.2)	16.3 (6.8)	6.5 (3.8)	1.1 (1.4)
P value		**0.0340***	**0.2885**	**0.1392**	**0.8693**	**0.0489**
HPV -ve	Male (67)	18.4 (7.9)	7.6 (5.5)	17.1 (7.3)	8.6 (3.5)	1.3 (1.5)
	Female (34)	20.1 (7.2)	9.0 (5.1)	16.7 (6.3)	9.1 (3.8)	1.0 (1.3)
P value		**0.2953**	**0.2186**	**0.7861**	**0.5114**	**0.3237**

Figure 4.5: Compares the dental status of genders with the same HPV status in OPC patients. *Unpaired t test with 95% confidence interval. Two-tailed p value < 0.05 is denoted as * with Bonferroni correction denoted as #*

Decade Total (+ve/-ve)	Teeth Present			Caries/Restoration			DMFT			Horizontal Bone Loss			Third Molar Present		
	Overall	HPV		Overall	HPV		Overall	HPV		Overall	HPV		Overall	HPV	
		+ve	-ve		+ve	-ve		+ve	-ve		+ve	-ve		+ve	-ve
25-34 2 (2/0)	22.5 (5.5)	22.5 (5.5)	-	3.5 (3.5)	3.5 (3.5)	-	8.0 (8.0)	8.0 (8.0)	-	1.5 (1.5)	1.5 (1.5)	-	1.0 (0.0)	1.0 (0.0)	-
35-44 17 (11/6)	25.4 (3.0)	25.5 (3.3)	25.0 (2.5)	6.5 (3.8)	6.5 (4.2)	6.5 (2.9)	9.1 (5.4)	9.0 (6.5)	9.3 (2.5)	5.7 (2.4)	5.3 (1.7)	6.5 (3.1)	1.8 (1.5)	1.9 (1.5)	1.7 (1.4)
45-54 97 (71/26)	24.4 (5.6)	23.5 (5.6)	23.3 (5.4)	8.3 (4.5)	8.7 (4.6)	7.2 (4.3)	12.6 (6.5)	13.0 (6.1)	11.8 (7.3)	6.2 (3.8)	5.3 (3.6)	8.6 (3.4)	1.6 (1.6)	1.6 (1.6)	1.5 (1.5)
55-64 121 (84/37)	21.6 (6.0)	23.3 (4.4)	17.8 (7.3)	10.1 (5.0)	10.6 (4.8)	9.1 (5.2)	16.4 (5.5)	15.2 (5.2)	19.2 (5.1)	7.7 (3.7)	6.8 (3.3)	9.7 (3.8)	1.4 (1.5)	1.6 (1.5)	1.0 (1.3)
65-74 64 (38/26)	17.5 (7.9)	18.7 (7.4)	15.8 (8.3)	9.0 (6.2)	9.6 (5.6)	8.0 (6.8)	19.1 (5.8)	18.5 (5.5)	20.0 (6.2)	8.0 (3.6)	8.0 (3.6)	8.1 (3.6)	1.1 (1.3)	1.1 (1.2)	1.1 (1.4)
75-84 15 (9/6)	14.8 (6.5)	14.3 (5.9)	15.5 (7.3)	9.0 (4.4)	9.8 (3.8)	7.8 (5.0)	22.0 (4.1)	23.1 (4.2)	20.3 (3.2)	8.2 (3.0)	7.9 (3.8)	8.7 (1.2)	0.9 (0.9)	0.8 (0.6)	1.2 (1.2)
P value	**0.0000** *#	**0.0000** *#	**0.0004** *#	**0.0161**	**0.0199**	**0.6313**	**0.0000** *#	**0.0000** *#	**0.0000** *#	**0.0007** *#	**0.0004** *#	**0.2019**	**0.1829**	**0.2481**	**0.5732**

Figure 4.6: Patients grouped by their age decade of presentation versus their dental status. Each decade is sub-categorised further based on the HPV status. *One-way ANOVA with 95% confidence intervals. p values with those <0.01 marked by an *. Bonferroni correction (p<0.003) denoted as #*

	HPV Status	Teeth Present	Caries/Restoration	DMFT	Horizontal Bone Loss	Third Molar Present
25-34	**HPV +ve (2)**	22.5 (5.5)	3.5 (3.5)	8.0 (8.0)	1.5 (1.5)	1.0 (0.0)
	HPV –ve (0)	-	-	-	-	-
P value		N/A	N/A	N/A	N/A	N/A
35-44	**HPV +ve (11)**	25.5 (3.3)	6.5 (4.2)	9.0 (6.5)	5.3 (1.7)	1.9 (1.5)
	HPV –ve (6)	25.0 (2.5)	6.5 (2.9)	9.3 (2.5)	6.5 (3.1)	1.7 (1.4)
P value		**0.7517**	**1.0000**	**0.9158**	**0.3131**	**0.7919**
45-54	**HPV +ve (71)**	23.5 (5.6)	8.7 (4.6)	13.0 (6.1)	5.3 (3.6)	1.6 (1.6)
	HPV –ve (26)	23.3 (5.4)	7.2 (4.3)	11.8 (7.3)	8.6 (3.4)	1.5 (1.5)
P value		**0.8754**	**0.1513**	**0.4181**	**0.0001*#**	**0.7823**
55-64	**HPV +ve (84)**	23.3 (4.4)	10.6 (4.8)	15.2 (5.2)	6.8 (3.3)	1.6 (1.5)
	HPV –ve (37)	17.8 (7.3)	9.1 (5.2)	19.2 (5.1)	9.7 (3.8)	1.0 (1.3)
P value		**0.0001*#**	**0.1253**	**0.0001*#**	**0.0001*#**	**0.0371**
65-74	**HPV +ve (38)**	18.7 (7.4)	9.6 (5.6)	18.5 (5.5)	8.0 (3.6)	1.1 (1.2)
	HPV –ve (26)	15.8 (8.3)	8.0 (6.8)	20.0 (6.2)	8.1 (3.6)	1.1 (1.4)
P value		**0.1479**	**0.3077**	**0.3129**	**0.9134**	**1.0000**
75-84	**HPV +ve (9)**	14.3 (5.9)	9.8 (3.8)	23.1 (4.2)	7.9 (3.8)	0.8 (0.6)
	HPV –ve (6)	15.5 (7.3)	7.8 (5.0)	20.3 (3.2)	8.7 (1.2)	1.2 (1.2)
P value		**0.7307**	**0.3937**	**0.1905**	**0.6295**	**0.4044**

Figure 4.7: Dental status of age decades with the opposing HPV status in OPC patients. *Unpaired t test with 95% confidence interval. Two-tailed p value < 0.05 is denoted as * with Bonferroni correction denoted as #*

	Current (106)	Ex-Smoker (131)	Never (83)	p value
Teeth Present	19.7 (7.5)	21.1 (7.1)	22.9 (5.0)	**0.0060*#**
Caries/Restoration	9.0 (5.6)	8.8 (4.9)	9.3 (4.8)	**0.7848**
DMFT	17.1 (7.1)	15.5 (6.3)	14.1 (6.5)	**0.0085*#**
Horizontal Bone Loss	8.7 (3.8)	6.8 (3.5)	5.8 (3.3)	**0.0000*#**
Third Molars Present	1.2 (1.4)	1.3 (1.5)	1.7 (1.5)	**0.0533**

Figure 4.8: Dental status of OPC based on smoking status. *One-way ANOVA with 95% confidence intervals. p values with those <0.05 marked by an * with Bonferroni correction denoted as #*

	Smoking Status	Teeth Present	Carious or restored teeth	DMFT	Horizontal bone loss	Third molar present
HPV +ve	**Current (49)**	20.8 (6.8)	9.2 (5.0)	16.0 (6.6)	8.5 (4.0)	1.1 (1.4)
	Ex-smoker (93)	22.3 (6.4)	9.7 (5.0)	15.2 (6.2)	6.0 (3.3)	1.4 (1.5)
	Never (73)	23.3 (4.6)	9.5 (4.8)	14.1 (6.3)	5.7 (3.2)	1.8 (1.5)
P value		**0.0777**	**0.8475**	**0.2496**	**0.0000*#**	**0.0332**
HPV -ve	**Current (55)**	19.2 (7.6)	9.0 (6.1)	17.7 (7.2)	9.0 (3.7)	1.3 (1.5)
	Ex-smoker (37)	18.5 (8.0)	6.8 (4.2)	16.2 (6.6)	8.7 (3.5)	1.1 (1.3)
	Never (9)	19.3 (6.6)	7.8 (4.4)	15.9 (6.7)	7.8 (2.7)	1.1 (1.4)
P value		**0.9030**	**0.1568**	**0.5307**	**0.6348**	**0.7809**

Figure 4.9: Compares the dental status of smoking status of patients at diagnosis with the same HPV status in OPC patients. *One-way ANOVA with 95% confidence intervals. p values with those <0.05 marked by an * with Bonferroni correction denoted as #*

	HPV Status	Teeth Present	Carious or restored teeth	DMFT	Horizontal bone loss	Third molar present
Current (104)	**HPV +ve (49)**	20.8 (6.8)	9.2 (5.0)	16.0 (6.6)	8.5 (4.0)	1.1 (1.4)
	HPV –ve (55)	19.2 (7.6)	9.0 (6.1)	17.7 (7.2)	9.0 (3.7)	1.3 (1.5)
P value		**0.2629**	**0.8563**	**0.2142**	**0.5094**	**0.4853**
Ex-smoker (130)	**HPV +ve (93)**	22.3 (6.4)	9.7 (5.0)	15.2 (6.2)	6.0 (3.3)	1.4 (1.5)
	HPV –ve (37)	18.5 (8.0)	6.8 (4.2)	16.2 (6.6)	8.7 (3.5)	1.1 (1.3)
P value		**0.0053***	**0.0023*#**	**0.4168**	**0.0001*#**	**0.2880**
Never (82)	**HPV +ve (73)**	23.3 (4.6)	9.5 (4.8)	14.1 (6.3)	5.7 (3.2)	1.8 (1.5)
	HPV –ve (9)	19.3 (6.6)	7.8 (4.4)	15.9 (6.7)	7.8 (2.7)	1.1 (1.4)
P value		**0.0217**	**0.3153**	**0.4241**	**0.0631**	**0.1344**

Figure 4.10: Smoking status of patient on diagnosis versus their dental status. Unpaired t test with 95% confidence interval. Two-tailed p value < 0.05 is denoted as *. Bonferroni correction (p<0.005) denoted as #

	Tonsil (166)	BOT (130)	OPC (Other) (24)	p value
Teeth Present	21.9 (6.7)	20.2 (7.0)	20.6 (7.1)	**0.1004**
Caries/Restoration	8.9 (5.3)	9.1 (4.9)	9.4 (5.4)	**0.8815**
DMFT	14.9 (7.1)	16.6 (6.1)	16.7 (6.2)	**0.0712**
Horizontal Bone Loss	6.9 (4.0)	7.5 (3.5)	6.9 (3.1)	**0.3721**
Third Molars Present	1.5 (1.5)	1.2 (1.4)	1.7 (1.6)	**0.1254**

Figure 4.11: Dental status of various micro sub-sites of OPC. *One-way ANOVA with 95% confidence intervals. p values with those <0.05 marked by an * with Bonferroni correction denoted as #*

	Sub-site	Teeth Present	Carious or restored teeth	DMFT	Horizontal bone loss	Third molar present
HPV +ve (215)	**Tonsil (123)**	23.1 (5.8)	9.1 (5.1)	13.9 (6.9)	6.1 (3.7)	1.5 (1.5)
	BoT (82)	20.1 (6.2)	9.8 (4.8)	16.6 (5.3)	7.2 (3.3)	1.3 (1.5)
	OPC (Other) (10)	23.2 (4.3)	11.5 (3.7)	16.1 (4.3)	5.3 (3.7)	1.6 (1.4)
P value		**0.0017*#**	**0.2551**	**0.0095***	**0.0551**	**0.6020**
HPV –ve (101)	**Tonsil (43)**	18.5 (7.8)	8.3 (5.7)	17.7 (7.0)	9.4 (3.7)	1.3 (1.4)
	BoT (45)	19.2 (7.7)	7.8 (4.9)	16.5 (7.0)	8.3 (3.7)	1.0 (1.3)
	OPC (Other) (13)	19.8 (7.1)	8.2 (6.1)	16.2 (6.8)	8.0 (2.0)	1.8 (1.7)
P value		**0.8381**	**0.9062**	**0.6603**	**0.2560**	**0.1800**

Figure 4.12: Compares the dental status of micro sub-sites of OPC at diagnosis with the same HPV status. One-way ANOVA with 95% confidence intervals. p values with those <0.05 marked by an * with *Bonferroni correction denoted as #*

	HPV Status	Teeth Present	Carious or restored teeth	DMFT	Horizontal bone loss	Third molar present
Tonsil (166)	**HPV +ve (123)**	23.1 (5.8)	9.1 (5.1)	13.9 (6.9)	6.1 (3.7)	1.5 (1.5)
	HPV –ve (43)	18.5 (7.8)	8.3 (5.7)	17.7 (7.0)	9.4 (3.7)	1.3 (1.4)
P value		**<0.0001*#**	**0.3919**	**0.0023*#**	**<0.0001*#**	**0.4452**
BoT (127)	**HPV +ve (82)**	20.1 (6.2)	9.8 (4.8)	16.6 (5.3)	7.2 (3.3)	1.3 (1.5)
	HPV –ve (45)	19.2 (7.7)	7.8 (4.9)	16.5 (7.0)	8.3 (3.7)	1.0 (1.3)
P value		**0.4747**	**0.0276**	**0.9175**	**0.0878**	**0.2612**
OPC (other) (23)	**HPV +ve (10)**	23.2 (4.3)	11.5 (3.7)	16.1 (4.3)	5.3 (3.7)	1.6 (1.4)
	HPV –ve (13)	19.8 (7.1)	8.2 (6.1)	16.2 (6.8)	8.0 (2.0)	1.8 (1.7)
P value		**0.1966**	**0.1469**	**0.9680**	**0.0354**	**0.7662**

Figure 4.13: Micro sub-sites of OPC versus dental status on presentation. Unpaired t test with 95% confidence interval. Two-tailed p value < 0.05 is denoted as *. Bonferroni correction (p<0.003) denoted as #

	I (6)	II (26)	III (27)	IV (261)	p value
Teeth Present	22. 0 (4.2)	18.5 (8.9)	22.8 (5.4)	21.2 (6.8)	**0.1378**
Caries/Restoration	8.8 (5.3)	9.3 (5.6)	10.3 (5.5)	8.8 (5.0)	**0.5242**
DMFT	14.7 (5.3)	18.7 (7.8)	15.5 (5.3)	15.4 (6.7)	**0.1171**
Horizontal Bone Loss	7.0 (3.4)	8.0 (4.5)	7.7 (3.7)	7.0 (3.7)	**0.5090**
Third Molars Present	1.3 (1.5)	1.2 (1.0)	1.3 (1.5)	1.4 (1.5)	**0.9117**

Figure 4.14: Dental status for stages of TNM7 in OPC patients. One-way ANOVA with 95% confidence intervals. p values with those <0.05 marked by an * with *Bonferroni correction denoted as #*

	I (26 – 23/3)	II (150 - 137/13)	III (68 – 55/13)	IV (72 – 0/72)	p value
Teeth Present	22.4 (6.5)	21.7 (6.4)	22.3 (5.8)	18.8 (7.7)	**0.0050*#**
Caries/Restoration	10.7 (6.1)	9.5 (4.7)	8.8 (5.2)	7.6 (5.3)	**0.0201***
DMFT	16.3 (6.9)	15.6 (6.4)	14.3 (6.2)	16.7 (7.2)	**0.1786**
Horizontal Bone Loss	6.2 (3.8)	6.5 (3.8)	7.6 (3.5)	8.5 (3.5)	**0.0008*#**
Third Molar Present	1.4 (1.3)	1.4 (1.4)	1.6 (1.6)	1.3 (1.5)	**0.6707**

Figure 4.15: Dental status for stages of TNM8 in OPC patients. Patients are presented as an overall total (number of HPV positive/number of HPV negative). *One-way ANOVA with 95% confidence intervals. p values with those <0.05 marked by an * with Bonferroni correction denoted as #*

Staging	Teeth Present	Carious or restored teeth	DMFT	Horizontal bone loss	Third molar present
I - TNM7 (6)	22.0 (4.2)	8.8 (5.3)	14.7 (5.3)	7.0 (3.4)	1.3 (1.5)
I - TNM8 (26)	22.4 (6.5)	10.7 (6.1)	16.3 (6.9)	6.2 (3.8)	1.4 (1.3)
P value	**0.8873**	**0.4880**	**0.5997**	**0.6398**	**0.8698**
II - TNM7 (26)	18.5 (8.9)	9.3 (5.6)	18.7 (7.8)	8.0 (4.5)	1.2 (1.0)
II - TNM8 (150)	21.7 (6.4)	9.5 (4.7)	15.6 (6.4)	6.5 (3.8)	1.4 (1.4)
P value	**0.0284**	**0.8460**	**0.0288**	**0.0725**	**0.4864**
III - TNM7 (27)	22.8 (5.4)	10.3 (5.5)	15.5 (5.3)	7.7 (3.7)	1.3 (1.5)
III - TNM8 (68)	22.3 (5.8)	8.8 (5.2)	14.3 (6.2)	7.6 (3.5)	1.6 (1.6)
P value	**0.7002**	**0.2153**	**0.3785**	**0.9019**	**0.4038**
IV - TNM7 (261)	21.2 (6.8)	8.8 (5.0)	15.4 (6.7)	7.0 (3.7)	1.4 (1.5)
IV - TNM8 (72)	18.8 (7.7)	7.6 (5.3)	16.7 (7.2)	8.5 (3.5)	1.3 (1.5)
P value	**0.0105**	**0.0761**	**0.1525**	**0.0022*#**	**0.6168**

Figure 4.16: Compares the dental status of equivalent stages of TNM7 and TNM8 in OPC patients. *Unpaired t test with 95% confidence interval. Two-tailed p value < 0.05 is denoted as * with Bonferroni correction denoted as #*

Stage/ TNM	HPV Status	Teeth Present	Carious or restored teeth	DMFT	Horizontal bone loss	Third molar present
I/7	**HPV +ve (3)**	20.0 (1.6)	7.7 (3.3)	15.7 (4.5)	9.0 (3.6)	2.0 (1.6)
I/7	**HPV – ve (3)**	24.0 (5.0)	10.0 (6.5)	13.7 (5.8)	5.0 (1.4)	0.7 (0.9)
P value		**0.2574**	**0.6138**	**0.6616**	**0.1473**	**0.2873**
II/7	**HPV +ve (12)**	20.8 (8.5)	10.4 (6.8)	17.6 (8.6)	5.6 (4.8)	1.4 (0.9)
II/7	**HPV – ve (13)**	17.5 (8.3)	8.6 (3.8)	18.9 (6.6)	10.2 (3.2)	1.1 (1.1)
P value		**0.3364**	**0.4174**	**0.6740**	**0.0093***	**0.4653**
III/7	**HPV +ve (14)**	24.9 (2.0)	10.9 (4.5)	14.0 (4.4)	6.0 (2.3)	1.4 (1.6)
III/7	**HPV – ve (13)**	20.5 (6.8)	9.7 (6.4)	17.2 (5.6)	9.5 (4.0)	1.3 (1.4)
P value		**0.0289**	**0.5758**	**0.1099**	**0.0094***	**0.8646**
IV/7	**HPV +ve (186)**	22.2 (6.0)	9.4 (4.8)	14.9 (6.3)	6.5 (3.6)	1.5 (1.5)
IV/7	**HPV – ve (72)**	18.8 (7.7)	7.6 (5.3)	16.7 (7.2)	8.5 (3.5)	1.3 (1.5)
P value		**0.0002*#**	**0.0092***	**0.0492**	**0.0001*#**	**0.3376**
I/8	**HPV +ve (23)**	22.2 (6.6)	10.8 (6.0)	16.7 (7.0)	6.4 (4.0)	1.3 (1.3)
I/8	**HPV – ve (3)**	24.0 (5.0)	10.0 (6.5)	13.7 (5.8)	5.0 (1.4)	0.7 (0.9)
P value		**0.6550**	**0.8311**	**0.4861**	**0.5592**	**0.4495**
II/8	**HPV +ve (137)**	22.1 (6.1)	9.6 (4.7)	15.3 (6.2)	6.2 (3.6)	1.4 (1.5)
II/8	**HPV – ve (13)**	17.5 (8.3)	8.6 (3.8)	18.9 (6.6)	10.2 (3.2)	1.1 (1.1)
P value		**0.0130**	**0.4583**	**0.0484**	**0.0002*#**	**0.4835**
III/8	**HPV +ve (55)**	22.7 (5.5)	8.6 (4.9)	13.6 (6.2)	7.1 (3.2)	1.7 (1.6)
III/8	**HPV – ve (13)**	20.5 (6.8)	9.7 (6.4)	17.2 (5.6)	9.5 (4.0)	1.3 (1.4)
P value		**0.2198**	**0.4956**	**0.0598**	**0.0237**	**0.4104**
IV/8	**HPV +ve (0)**	-	-	-	-	-
IV/8	**HPV – ve (72)**	18.8 (7.7)	7.6 (3.3)	16.7 (7.2)	8.5 (3.5)	1.3 (1.5)
P value		**N/A**	**N/A**	**N/A**	**N/A**	**N/A**

Figure 4.17: Dental status of TNM7 and TNM8 staging based on HPV status. Unpaired t test with 95% confidence interval. Two-tailed p value < 0.05 is denoted as * Bonferroni correction ($p<0.0025$) denoted as #

	Stage/ TNM	Teeth Present	Carious or restored teeth	DMFT	Horizontal bone loss	Third molar present
HPV+ve	**I/7 (3)**	20.0 (1.6)	7.7 (3.3)	15.7 (4.5)	9.0 (3.6)	2.0 (1.6)
	I/8 (23)	22.2 (6.6)	10.8 (6.0)	16.7 (7.0)	6.4 (4.0)	1.3 (1.3)
P value		**0.5769**	**0.3944**	**0.8134**	**0.2964**	**0.3989**
	II/7 (12)	20.8 (8.5)	10.4 (6.8)	17.6 (8.6)	5.6 (4.8)	1.4 (0.9)
	II/8 (137)	22.1 (6.1)	9.6 (4.7)	15.3 (6.2)	6.2 (3.6)	1.4 (1.5)
P value		**0.4949**	**0.5875**	**0.2353**	**0.5913**	**1.0000**
	III/7 (14)	24.9 (2.0)	10.9 (4.5)	14.0 (4.4)	6.0 (2.3)	1.4 (1.6)
	III/8 (55)	22.7 (5.5)	8.6 (4.9)	13.6 (6.2)	7.1 (3.2)	1.7 (1.6)
P value		**0.1475**	**0.1160**	**0.8213**	**0.2319**	**0.5332**
	IV/7(186)	22.2 (6.0)	9.4 (4.8)	14.9 (6.3)	6.5 (3.6)	1.5 (1.5)
	IV/8 (0)	-	-	-	-	-
P value		**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
HPV - ve	**I/7 (3)**	24.0 (5.0)	10.0 (6.5)	13.7 (5.8)	5.0 (1.4)	0.7 (0.9)
	I/8 (3)	24.0 (5.0)	10.0 (6.5)	13.7 (5.8)	5.0 (1.4)	0.7 (0.9)
P value		**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**
	II/7 (13)	17.5 (8.3)	8.6 (3.8)	18.9 (6.6)	10.2 (3.2)	1.1 (1.1)
	II/8 (13)	17.5 (8.3)	8.6 (3.8)	18.9(6.6)	10.2 (3.2)	1.1 (1.1)
P value		**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**
	III/7 (13)	20.5 (6.8)	9.7 (6.4)	17.2 (5.6)	9.5 (4.0)	1.3 (1.4)
	III/8 (13)	20.5 (6.8)	9.7 (6.4)	17.2 (5.6)	9.5 (4.0)	1.3 (1.4)
P value		**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**
	IV/7 (72)	18.8 (7.7)	7.6 (5.3)	16.7 (7.2)	8.5 (3.5)	1.3 (1.5)
	IV/8 (72)	18.8 (7.7)	7.6 (5.3)	16.7 (7.2)	8.5 (3.5)	1.3 (1.5)
P value		**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**

Figure 4.18: Compares the dental status of equivalent stages of TNM7 and TNM8 based on the same HPV status in OPC patients. *Unpaired t test with 95% confidence interval. Two-tailed p value < 0.05 is denoted as * with Bonferroni correction denoted as #*

		TILs	
		High (140)	**Low (17)**
Gender	Male	108	17
	Female	32	0
Age	18-34	1	0
	35-44	9	0
	45-54	47	4
	55-64	52	5
	65-74	23	8
	75-84	8	0
Smoking status	Never smoked	43	5
	Ex-smoker	63	7
	Current smoker	34	5
Sub-site	Tonsil	76	6
	BOT	53	10
	Oropharynx (other)	11	1
TNM7 Stage	I	1	0
	II	6	3
	III	7	2
	IV	126	12
TNM8 Stage	I	12	5
	II	88	6
	III	40	6

Figure 4.19: Demographic breakdown of the current cohort based on TILs-high and TILs-low grading.

TILs	Teeth Present	Caries/Restorations	DMFT	Horizontal Bone Loss	Third Molars Present
High (140)	20 (7.4)	7.6 (4.9)	15.6 (7.2)	7.2 (4.1)	1.5 (1.4)
Low (17)	19.0 (7.8)	7.4 (4.9)	16.1 (5.9)	8.2 (4.2)	1.2 (1.4)
p value	***0.6016***	***0.8739***	***0.7836***	***0.3450***	***0.4054***

Figure 4.20: Mean values for each dental domain with standard deviation in brackets for the total cohort based in TILs grading. *Unpaired t-test with 95% confidence intervals with p values with those <0.05 marked by an *.*

Demographic	TILs	Teeth Present	Caries/Restoration	DMFT	Horizontal Bone Loss	Third Molars Present
Male (125)	High (108)	20.6 (6.9)	7.7 (4.9)	15.0 (7.0)	7.3 (4.3)	1.7 (1.5)
	Low (17)	19.0 (7.8)	7.4 (4.9)	16.1 (5.9)	8.2 (4.2)	1.2 (1.4)
p value		*0.3843*	*0.8149*	*0.5404*	*0.4226*	*0.2000*
Female (32)	High (32)	17.9 (8.6)	7.5 (4.8)	17.6 (7.7)	6.7 (3.3)	1.1 (1.2)
	Low (0)	-	-	-	-	-
p value		*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
p value		***0.1700***	***0.9595***	***0.1822***	***0.4757***	***0.0742***

Figure 4.21: Mean values for each dental domain with standard deviation in brackets for each gender which is further sub-divided based on their TIL grading. *Statistical analysis within each gender is via unpaired t-test with 95% confidence intervals while overall population* statistical analysis is via *One-way ANOVA with 95% confidence intervals. In both tests p values with those <0.05 marked by an *. Bonferroni correction (p<0.005) denoted as #.*

Demographic	TIL	Teeth Present	Caries/Restoration	DMFT	Horizontal Bone Loss	Third Molars Present
18-34 (1)	High (1)	24.0 (0.0)	4.0 (0.0)	8.0 (0.0)	1.0 (0.0)	0.0 (0.0)
	Low (0)	-	-	-	-	-
p value		*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
35-44 (9)	High (9)	25.1 (2.6)	8.1 (2.5)	10.7 (2.8)	6.9 (4.7)	1.4 (1.6)
	Low (0)	-	-	-	-	-
p value		*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
45 – 54 (51)	High (47)	21.2 (7.0)	6.4 (4.1)	13.3 (6.7)	7.5 (4.0)	2.0 (1.4)
	Low (4)	17.8 (6.8)	8.3 (4.7)	18.5 (3.9)	11.5 (2.1)	0.8 (0.8)
p value		*0.3548*	*0.3825*	*0.1346*	*0.0552*	*0.0992*
55-64 (57)	High (52)	19.7 (7.1)	8.8 (5.2)	17.1 (7.4)	7.3 (4.0)	1.5 (1.4)
	Low (5)	22.6 (7.4)	6.8 (5.6)	11.8 (6.2)	6.6 (4.5)	0.4 (0.8)
p value		*0.3883*	*0.4176*	*0.1277*	*0.7127*	*0.0909*
65-74 (31)	High (23)	16.9 (7.3)	6.9 (5.2)	18.0 (7.0)	7.3 (3.6)	0.8 (1.2)
	Low (8)	17.4 (7.9)	7.4 (4.8)	17.5 (5.2)	7.6 (3.9)	1.9 (1.6)
p value		*0.8712*	*0.8131*	*0.8551*	*0.8437*	*0.0496**
75-84 (8)	High (8)	17.9 (10.6)	8.9 (6.2)	19.0 (6.6)	4.6 (4.5)	1.1 (1.2)
	Low (0)	-	-	-	-	-
p value		*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
p value		***0.1129***	***0.4255***	***0.0072****	***0.2131***	***0.0177****

Figure 4.22: Mean values for each dental domain with standard deviation in brackets for each age decade which is further sub-divided based on their TIL grading. *Statistical analysis within each age decade is via unpaired t-test with 95% confidence intervals while overall population* statistical analysis is via *One-way ANOVA with 95% confidence intervals. In both tests p values with those <0.05 marked by an *. Bonferroni correction (p<0.0025) denoted as #*

Demographic	TILs	Teeth Present	Caries/Restoration	DMFT	Horizontal Bone Loss	Third Molars Present
Never smoked (48)	High (43)	21.5 (7.1)	7.0 (4.4)	13.4 (6.5)	6.7 (3.9)	1.6 (1.5)
	Low (5)	20.4 (9.1)	6.4 (5.5)	13.6 (8.5)	8.0 (4.7)	1.2 (1.6)
p value		*0.7511*	*0.7794*	*0.9499*	*0.4924*	*0.5775*
Ex-smoker (70)	High (63)	19.2 (8.1)	8.0 (5.4)	16.8 (7.6)	7.3 (4.2)	1.6 (1.5)
	Low (7)	20.3 (6.9)	8.4 (4.3)	15.6 (3.6)	7.4 (4.5)	1.6 (1.5)
p value		*0.7311*	*0.8507*	*0.6826*	*0.9528*	*1.0000*
Current smoker (39)	High (34)	19.6 (6.1)	7.7 (4.4)	16.2 (6.8)	7.4 (4.0)	1.3 (1.3)
	Low (5)	15.8 (6.7)	7.0 (4.9)	19.2 (3.6)	9.6 (2.4)	0.6 (0.8)
p value		*0.2063*	*0.7448*	*0.3437*	*0.2415*	*0.2519*
p value		***0.5180***	***0.8933***	***0.1540***	***0.7465***	***0.6494***

Figure 4.23: Mean values for each dental domain with standard deviation in brackets for smoking status which is further sub-divided based on their TIL grading. *Statistical analysis within each status is via unpaired t-test with 95% confidence intervals while overall population* statistical analysis is via *One-way ANOVA with 95% confidence intervals. In both tests p values with those <0.05 marked by an *. Bonferroni correction (p<0.0025) denoted as #.*

Demographic	TILs	Teeth Present	Caries/Restoration	DMFT	Horizontal Bone Loss	Third Molars Present
Base of tongue	High (53)	19.8 (7.4)	7.3 (5.3)	15.4 (7.3)	7.3 (4.1)	1.3 (1.5)
	Low (10)	19.6 (8.6)	8.5 (4.5)	16.3 (5.8)	6.7 (4.4)	0.9 (1.3)
p value		*0.9393*	*0.5050*	*0.7143*	*0.6761*	*0.4337*
Oropharyngeal (other)	High (11)	19.8 (8.6)	6.0 (4.7)	14.2 (8.7)	5.7 (3.3)	1.8 (1.5)
	Low (1)	23.0 (0.0)	11.0 (0.0)	16.0 (0.0)	14.0 (0.0)	2.0 (0.0)
p value		*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Tonsil	High (76)	20.1 (7.2)	8.1 (4.6)	15.9 (6.9)	7.3 (4.1)	1.6 (1.4)
	Low (6)	17.3 (6.7)	5.0 (4.9)	15.7 (6.7)	9.8 (2.3)	1.5 (1.6)
p value		*0.3599*	*0.1175*	*0.9456*	*0.1456*	*0.8679*
p value		***0.9634***	***0.4516***	***0.9825***	***0.2238***	***0.6059***

Figure 4.24: Mean values for each dental domain with standard deviation in brackets for each varying OPC anatomical sites which is further sub-divided based on their TIL grading. *Statistical analysis within each OPC sub-site is via unpaired t-test with 95% confidence intervals while overall population* statistical analysis is via *One-way ANOVA with 95% confidence intervals. In both tests p values with those <0.05 marked by an *. Bonferroni correction (p<0.0033) denoted as #.*

TNM	TILs	Teeth Present	Caries/ Restoration	DMFT	Horizontal Bone Loss	Third Molars Present
7 - I (1)	High (1)	17.0 (0.0)	12.0 (0.0)	23.0 (0.0)	11.0 (0.0)	2.0 (0.0)
	Low (0)	-	-	-	-	-
p value		*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
7 - II (9)	High (6)	19.3 (8.7)	11.1 (5.3)	20.0 (4.1)	6.7 (4.8)	1.7 (1.2)
	Low (3)	18.7 (6.8)	7.3 (3.9)	16.7 (3.3)	10.3 (2.9)	1.0 (0.8)
p value		*0.9205*	*0.3127*	*0.2691*	*0.2794*	*0.3983*
7 - III (9)	High (7)	20.4 (9.2)	9.9 (7.4)	17.6 (6.9)	5.9 (2.6)	2.0 (1.2)
	Low (2)	11.0 (8.0)	4.0 (2.0)	20.0 (7.0)	7.0 (4.0)	1.0 (1.0)
p value		*0.2357*	*0.3211*	*0.6781*	*0.6441*	*0.3232*
7 - IV (138)	High (126)	20.0 (7.2)	7.3 (4.6)	15.2 (7.3)	7.2 (4.1)	1.5 (1.5)
	Low (12)	20.4 (7.2)	8.0 (5.2)	15.3 (6.0)	7.9 (4.3)	1.3 (1.6)
p value		*0.8544*	*0.6192*	*0.9634*	*0.5745*	*0.6614*
p value		***0.7668***	***0.2948***	***0.5152***	***0.7124***	***0.9346***
8 - I (17)	High (12)	19.3 (9.0)	9.4 (5.4)	18.3 (5.9)	6.6 (4.2)	1.6 (1.0)
	Low (5)	15.6 (8.2)	6.0 (3.6)	18.0 (5.4)	9.0 (3.7)	1.0 (0.9)
p value		*0.4416*	*0.2194*	*0.9235*	*0.2857*	*0.2654*
8 - II (94)	High (88)	19.7 (7.1)	8.1 (5.1)	16.4 (7.1)	7.4 (3.8)	1.5 (1.5)
	Low (6)	23.3 (5.3)	8.0 (4.9)	12.0 (6.3)	7.7 (4.7)	2.2 (1.7)
p value		*0.2269*	*0.9630*	*0.1430*	*0.8541*	*0.2753*
8 - III (46)	High (40)	20.8 (7.4)	6.0 (3.6)	13.1 (7.1)	6.8 (4.6)	1.7 (1.5)
	Low (6)	17.5 (7.6)	8.0 (5.5)	18.5 (3.3)	8.2 (3.8)	0.3 (0.7)
p value		*0.0732*	*0.2434*	*0.0755*	*0.4826*	*0.0307**
p value		***0.4957***	***0.1704***	***0.0445****	***0.8170***	***0.2318***

Figure 4.25: Mean values for each dental domain with standard deviation in brackets for the staging based on TNM7 & TNM8 which are further sub-divided based on their TIL grading. *Statistical analysis within each stage is via unpaired t-test with 95% confidence intervals while overall population* statistical analysis *per TNM classification* is via *One-way ANOVA with 95% confidence intervals. In both tests p values with those <0.05 marked by an *. Bonferroni correction (p<0.0025) is denoted as #.*

4.5 Discussion

Historically, OPC was associated with traditional HNC risk factors such as tobacco exposure and excessive alcohol use. However, most recently a new risk factor for this disease has emerged and is starting to dominate the HNC landscape (Louie et al., 2015). This HPV positive population is male dominated, peaks in 55-65Y (Chaturvedi et al., 2008) age group and many do not have smoking and alcohol habits. Hence planning dental care for OPC requires new considerations for the different sub-groups with this tumour site.

When tailoring pre-RT dental care to an individual patient it is useful to be aware of factors that have contributed to their dental disease as one might assume they will continue post-RT. The stereotype of a heavy drinking and smoking individual with neglected dental health and irregular dental attendance does not normally apply to HPV positive OPC patients (Vinod. Patel et al., 2020). The latter have better dentitions to other HNC sub-sites (Vinod. Patel et al., 2020; V. Patel et al., 2020). This is a stark contrast to only 3 decades ago where Maier et al reported that pre-RT dental status was worst in OPC patients and is a reflection to the emergence of HPV positive related disease (Maier et al., 1993).

Consequently, HPV status is important to dental treatment planning but until now has not been subject of investigation. The present data show there is a significant difference in the dental status between HPV positive and negative patients. Overall the positive group presented on average with 3 additional teeth as well as both lower HBL scores and DMFT scores and more caries/restored teeth. HPV positive patients have a healthier, more complex dentition with the proviso the dentition is more vulnerable. Now add to the equation the gradual dental decay that will occur over the next 30 years (Ang et al., 2010; Ang & Sturgis, 2012; Li et al., 2003; O'Sullivan et al., 2012) with the attendant risk of ORN increasing over time (Caparrotti et al., 2017). Also, the ever-present medico-legal issues that follow the disease.

One prevalent approach to counter ORN has been the radical practice of mass prophylactic molar extractions at the pre-RT assessment. Surprisingly, this approach does not guarantee avoidance of ORN and ironically can increase in risk of this disease (N. M. Beech et al., 2017). Also, this invasive approach creates a dentition below the accepted functional threshold (V. Patel et al., 2020). Though this blanket strategy of mass

prophylactic extractions is employed with good clinical intentions, it undervalues the role of the dentition and detracts from QoL (N. Beech et al., 2016). Of all the HNC patients the dilemma between radical extraction and a conservative approach is most vivid in the HPV positive sub-group. These patients present with more teeth and with more complex restorations. Therefore, ironically, engagement with a traditional dental service leads to a remedial dentition with a lack of function on the background of long-term survivorship.

Though improved prognosis has been consistently shown in HPV positive OPC compared with HPV negative tumors (Ang et al., 2010; Fakhry et al., 2008; Lindquist et al., 2007; Rischin et al., 2010; Weinberger et al., 2006), 20% of patients with HPV positive OPC are estimated to die of their disease (Mirghani & Blanchard, 2018). Hence, not all HPV positive patients share the same favourable outcome commonly associated with this tumour type. More recently, the presence of TILs within tumors has been identified as a powerful prognostic factor and has been used in colorectal cancer, ovarian cancer, and other malignancies (Anitei et al., 2014; Galon et al., 2006; Galon et al., 2014; Zhang et al., 2003) with potential to improve prognostic stratification compared with TNM staging (Anitei et al., 2014; Galon et al., 2006; Galon et al., 2014; Mlecnik et al., 2011; Zhang et al., 2003). The use of TILs has been extended to HNC and particularly in OPC with studies consistently finding high TILs counts to have better overall and disease-free survival (Badoual et al., 2013; Balermpas et al., 2016; Dahlin et al., 2011; Denkert et al., 2010; Ducray et al., 2010; Gooden et al., 2011; Grabenbauer et al., 2006; Keck et al., 2015; Matlung et al., 2016; Näsman et al., 2009; Nedergaard et al., 2007; Nordfors et al., 2013; Oguejiofor et al., 2015; Ward et al., 2014). Specifically, for HPV positive OPC, TILs stratification showed stark variation for survival (Ward et al., 2014). When tumours were sub-divided as TILs-high, TILs-moderate, TILs-low the 3-year survival was 96%, 76% and 59% respectively (Ward et al., 2014). Interestingly, it was concluded that TILs-low HPV positive OPC patients have a similar survival to HPV negative patients, which was 56% at 3 years. It has therefore been proposed that a prognostic model based on low TILs levels, heavy smoking, and late T stage is extremely effective at identifying a group of HPV-positive patients with poor survival (Ward et al., 2014). The clinical value of TILs classification is that together with other factors it adds to the prognostic picture that informs the dental oncologist and allows them to tailor advice to the individual.

In the current study comparing the dentition of TILs-high and TILs-low did not identify

any statistical difference. Observationally the dental status was very similar with TILs-low having marginally worse dental scores in each dental criterion. The presumption is that TILs does not have a direct impact on dental status within the HPV positive OPC group due its role in tumour biology rather than direct influence such as traditional risk factors. Hence TILs are more likely to be a function of the tumour rather than the host. Though not a direct influencer in the dental status it still has value for the dental sector. Its input in survival outcomes is a key consideration during dental treatment planning. Furthermore, there is a move to treatment de-escalation, but patient selection criteria still needs to be identified. A TILs-low OPC may disqualify patients from de-escalation regimes, but since there are no differences in dental status the elevated ORN risk in this tumour group will continue to exist.

4.5.1 Gender

The rise of OPC has impacted both genders worldwide in economically developed countries (Chaturvedi et al., 2013) but is more prevalent in the male population (Chaturvedi et al., 2013; Chaturvedi et al., 2011). There is no difference in their basic dental status highlighting gender itself does not influence dentition in OPC. In the current study males accounted for 72% (172/239) of the HPV positive cases and had more complex dentition. This inflated population within OPC identified them as a prevalent and vulnerable group. Interestingly, regardless of HPV status, females had similar number of teeth. However, it appears HPV negative patients are retaining these teeth on a background of worsening periodontal disease compared to their counterparts. The implications for HPV negative females who continue to smoke is that they present a risk of ORN because of the presence of periodontally involved teeth. The obvious approach would be pre-RT extractions but the evidence shows >8 pre-RT dental extractions, HPV negative status, female gender and positive smoking status were associated reduced QoL (N. Beech et al., 2016). This creates a complex scenario to manage and balance the dentition pre-RT. Though in the current study there were no TILs-low females, if the concept that TILs-low are equivalent to HPV negative patients there may be some overlap here to consider.

A different challenge presents in the male HPV positive patients who have a good prospect of cure and normally have reasonable dental status at presentation but if prophylactic pre-RT extractions are undertaken then there is a negative impact on function. Yet the

presence of multiple teeth over time raises the threat of radiation caries and of ORN from extractions. It is therefore imperative that meticulous long-term dental care is essential in this group of patients.

When considering HPV positive patients sub-divided by TILs no discernible difference was evident between or within genders. However, HPV related OPC being more prevalent in the male population (Chaturvedi et al., 2013; Chaturvedi et al., 2011), survival of these patients will require dedicated long-term dental care. Identifying these patients with TILs and overlaying their dental status will allow the dental oncologist to consider longevity of the dentition based on expected survivorship.

4.5.2 Age

A major concern regarding OPC is that the disease is being seen in younger patients compared to other HNCs (Chaturvedi et al., 2013). The median age for OPC is 63 years (Chaturvedi et al., 2008) which drops to 58 years for HPV positive group (Chaturvedi et al., 2011). In the current study the majority of patients fell between 55-64 years old. The relevance of age is that the 2009 Adult Dental Health Survey (ADHS) reported adults <65 years old rarely lose all their natural teeth (ADHS, 2009). This is supported by the results in the current study where HPV positive patients had 23 teeth compared to 18 teeth in HPV negative patients ($p=0.0001$). The clinical challenge is that many of these individuals have extensive and sometimes complex dental restorations ('heavy metal generation') that require very careful dental maintenance. Prophylactic pre-RT extraction of the teeth is not the solution if the number of dental units falls below 21 deemed to be the threshold of a functional dentition by the ADHS (ADHS, 2009). The complexity of the problem is illustrated when it is realized most of these patients have on average 10 carious/restored teeth, mild HBL and at least one third molar in situ.

DMFT scores increase with age as might be expected and is irrespective of HPV status. Interestingly, only the 55-64 age group showed difference in DMFT between HPV statuses ($p=0.0001$). HPV positive patients have more teeth so their larger DMFT score is likely to be associated with carious/restored teeth whereas as for the HPV negative patients it is due to a lack of teeth. This theme is continued with HBL status. There is increasing bone loss with age in the positive group and less so in the negative group. This is because in the negative cohort the bone was already lost prior to developing OPC probably due to

smoking habits. The current study identified DMFT scores and third molars as significant dental criteria with increasing age. However, no trend between TILs-high and TILs-low can be clearly identified and therefore the significance is strongly related to recognised increase in dental disease through exposure over time.

Age related tooth loss in the subsequent 2 decades after age 55-64, identified 9 teeth less in the HPV positive and only 2 in the HPV negative patients. These events expose patients to the risk of ORN and emphasize the need for careful dental maintenance and explains the slowly increasing rate of ORN. Considering TILs, this would continue to be a concern in the TILs-high sub-group for dental treatment planning. In contrast, low survivorship versus the risk of ORN provides a differing dental conundrum in TILs-low patients. Paradoxically the HPV negative patient presents with less teeth, lose less teeth post RT and have lower survivorship. Hence, this sub-group's risk of ORN is commensurately less.

4.5.3 Smoking

It is well established that smoking impacts negatively on dental health (Tezal et al., 2013) and this was evident in the present study population with increase HBL (p=0.0000) and increasing DMFT (p=0.0085) score based on smoking status. The net result is less teeth with the effect extending into ex-smokers when compared to non-smokers respectively (19.7 vs 21.1 vs 22.9, p=0.006).

The public awareness of smoking as a health hazard has led to reduction in tobacco consumption and with it a decline in smoking related HNCs (Blot et al., 1994; CDC, 2013; Franceschi et al., 2000). In contrast, OPC incidence has increased over the last 20 years in several developed countries related to HPV (Auluck et al., 2010; Blomberg et al., 2011; Chaturvedi, 2012; Chaturvedi et al., 2013; Chaturvedi et al., 2008; Gillison, Alemany, et al., 2012; Hammarstedt et al., 2006; A. M. Hong et al., 2010; Marur et al., 2010; J. Mork et al., 2010; Ramqvist & Dalianis, 2010; Reddy et al., 2010) and many of these patients do not smoke. Most series show about 30% are NS in the HPV positive group compared with less than 5% in the HPV negative group (A. M. Hong et al., 2010). Our cohort reflects similar figures to this with 34% NS in the HPV positive group compared to 9% in the HPV negative group confirming the association with tobacco and alcohol use and poor oral hygiene (D'Souza et al., 2007; Gillison et al., 2008).

In the current study smoking status based on TILs classification was not significant even though observationally, TILs-high patients appeared to have better teeth than TILs-low patients. The comparison of TILs-low to HPV negative patients has suggested shared risk factors such as heavy smoking. However, currently no significant association between heavy smoking and low TILs levels, has been consistently identified (Ward et al., 2014).

4.5.4 Sub-site

In the HPV positive patients tonsillar and OPC (other) had a better dentition than BOT patients. This could not be attributed to mean age, which was similar in all 3 groups (57.5-59.5 years). The impact of smoking is likely to explain these differences in dentition. Though NS dominated the HPV positive group while the reverse was true for HPV negative group this trend was seen to a lesser degree in the BOT group.

Dividing these groups further by TILs did not show any statistical difference in dentition. Limited numbers particularly in certain groups means even observational assessment is limited as a small group are unlikely to be truly representative.

4.5.5 Staging

The theme repeatedly identified is that HPV positive patients have more teeth and a better standard of dental health is further evident in the staging of OPC. When the study group were staged by the TNM7 system the vast majority of tumours (n= 261, 81.6%) were stage IV of which 189 patients were HPV positive. Re-staging in accordance to TNM8 meant stage IV made up only 22.8% (n=72) with stage II now the most dominant group. In TNM7 paradoxically the stage IV cases had a better dental status as they dominated with large numbers of HPV positive cases. Currently, TNM7-stage IV disease is treated with both chemo-RT involving the neck bilaterally regardless of HPV status leading to large parts of the dento-alveolar bone being incorporated with the radiation field, often above the 40Gy threshold for ORN. The recognition of favourable outcomes in HPV positive patients has led to the proposal of the new TNM8 which is subsequently stimulating research into de-escalation (ClinicalTrials.gov, 2019; ISRCTN, 2018) of treatment in particular groups.

Re-staging patients in accordance to TNM8 showed that stages I-III were now populated with HPV positive cases had a similar number of teeth (22.4/21.7/22.3) compared 18.8

in stage IV populated only with HPV negative disease. The trend was reflected for HBL severity scores. There has been no proposed change to the HPV negative group for treatment de-escalation. Hence, stage IV HPV negative patient will continue to receive extensive chemo-RT on the background of periodontal disease and subsequent risk of ORN. However, arguably, ORN is a late effects complication and commonly seen in the posterior mandible. With an overall 3-year survival of 57.1% (Ang et al., 2010), a lack of posterior dentition lost through progressive periodontal disease these patients may have a lesser ORN risk rate. This is in contrast to their HPV positive colleagues with higher survival, a more complex dentition including more posterior teeth and time for radiation induced fibrotic late effects to develop.

A weak significance was seen for DMFT scores in TNM8 but no reliable trend was evident. Similarly, a weak significance was also seen in stage III between TILs-high and TILs-low for the presence of third molars. Though TILs classification is unlikely to have a direct impact on third molar presence this finding is potentially one of significance. With evidence that third molars will succumb to dental disease, their position in the jaws being directly within RT beam pathways for the oropharynx and hence receiving near total tumour dose they are deemed to increase the risk of ORN in OPC patients.

4.6 Conclusion

The rise in incidence of OPC with its improved survivorship, particularly, in HPV positive patients needs to be complimented by a functional dentition. The current study demonstrates that the HPV positive cohort have a complex dentition presenting new challenges to the dental oncologist. The dental intricacies identified in OPC patients provide an explanation for the elevated ORN rate seen in this tumour group.

Though TILs does not have a direct impact on the dentition its does help risk stratify HPV positive OPC patients and may have role in future pre-RT dental planning. It is likely that TILs-low OPC is not the same as TILs-high OPC with each group possibly needing differing treatment such escalating treatment in the former and de-escalating treatment in the latter. Hence, the ability to balance cancer outcome with dental treatment planning is a theme of this thesis. Functional ideals have to be balanced against the risk of future ORN. TILs are easy to produce and be available at the time of pre-RT dental assessment. Though no definitive conclusions can be made from this study for TILs, some early insight

suggests that it may have a role in better informing dental treatment planning. It requires further validation and acceptance within the oncology sector for routine use.

4.7 Limitations

4.7.1 Dental status

The limitation stated in section 3.7 for the preceding dental status study remain applicable and identical for the current dental study too.

4.7.2 TILs

The small number of TILs-low patients in the study remains a limitation for analysis when comparing to TILs-high. The inability to show a difference in the dental status does not necessarily mean one does not exist and should be repeated with a larger cohort of patients of near equal number of TILs-high and TILs-low patients for a definitive outcome.

Chapter 5

Radiation doses to the teeth and alveolar bone in oropharyngeal cancer patients following intensity modulated radiation treatment

5.1 Introduction

It is highly recommended that HNC patients undergoing RT have a dental assessment prior to commencing (NICE, 2004; RCS, 2019; RD-UK, 2016). However, the intricacies to achieve 'dental fitness' are not standardized. Extraction of unrestorable teeth and those with dental pathology judged to be of poor prognosis is uncontested. However, pre-RT dental extraction of sound teeth remains a contentious approach with conflicting ORN rates (2.6.2.1 Pre-radiotherapy dental extractions, Figure 2.5) reported in the literature (N. M. Beech et al., 2017; Chang et al., 2007; Moon et al., 2017; Studer et al., 2006; Wahl, 2006). Prophylactic dental extraction continues to be employed, particularly in OPC (Caparrotti et al., 2017) due to concern of elevated RT doses to the jaws (A. Owosho et al., 2017). However, pre-RT dental care is compromised by the need to be deemed to be 'dentally fit' within a two week window between diagnosis to allow commencement of RT for a better treatment response (Grønhøj et al., 2018).

The dental status of the OPC group (Vinod. Patel et al., 2020; V. Patel et al., 2020) has been analysed and identified as risk factor (Kojima et al., 2017) for ORN in the previous chapters. At the pre-RT dental assessment, the HPV positive group present with more teeth compared to HPV negative patients (Vinod. Patel et al., 2020) (Chapter 4). Furthermore, their dentition was deemed to be better and more complex too maintain (Vinod. Patel et al., 2020) (Chapter 4). This provides ample opportunity for post-RT dental disease to progress and ORN to evolve. Additional risk factors of smoking (Tsai et al., 2013), periodontal disease (Schuurhuis et al., 2011) and pre-RT dental extractions (N. Beech et al., 2016; Chang et al., 2007) heightens the risk of ORN.

Limitations on time to treatment and lack of knowledge of RT dose to individual dental units produce poorly tailored dental treatment plans. Over treatment, particular with dental extractions in pre-RT OPC patients needs re-evaluating as this practice did not improve QoL but made it worse (N. Beech et al., 2016).

The risk of ORN is driven mainly by two factors; the health of the dentition and RT doses. These are the responsibility of two different clinical disciplines. An integrated assessment and approach are not the norm. The current observational study focuses on the actual radiation exposure the teeth received. The objective is to help inform and guide the pre-RT dental assessment process. The threshold for ORN is > 40 Gy (Cooper, 2003) and pre-RT dental treatment planning focuses around this perceived dose to the dentition. The posterior dentition is assumed to exceed the 40Gy barrier and raises the argument for

prophylactic extraction. The introduction of IMRT through its multi-beams delivery has meant a more diffuse delivery of radiation to local structures (Rosenthal et al., 2008). However, paradoxically with IMRT dose to the anterior mandible can commonly exceed 40Gy (Rosenthal et al., 2008). Incisor teeth are always retained and in practice are at little risk of ORN. This contradicts the tenants of current practice. An additional risk factor for ORN is adjuvant chemotherapy. The addition of chemotherapy to RT is estimated to be the equivalent of an extra 10 Gy.

Hence, to appropriately treatment plan the OPC patient a better understanding of the doses of radiation to the individual dental units is essential. This has led to a series of studies reporting on the dose to the dento-alveolar segments. Unfortunately, preceding studies have shown significant limitations. Commonly, they have not distinguished between different HNC sub-sites, clustered tumour and nodal statuses together or consisted of limited patient numbers. Furthermore, the dental contours have been grouped into segmented areas of the jaw rather than individual teeth.

The current observational study explores the radiation dose to dental units in OPC cases and is the largest study of its kind to date. The intention of this study is to establish RT dose maps to assist dental treatment planning both pre- and post-RT.

5.2 Methodology

5.2.1 Ethics

19/EE/0224 - Dental status, radiotherapy doses and subsequent implications in head and neck cancer patients - A retrospective cohort study

5.2.2 Patient population

The electronic log of biopsy confirmed OPC was searched with date limits of March 2011 until March 2017. The earlier date related to the introduction and routine use of IMRT for HNC within the Trust (GSTFT). The inclusion criteria included all patients diagnosed with OPC treated primarily with IMRT with a curative intent. OPC included the base of the tongue, the inferior surface of the soft palate and uvula, the anterior and posterior tonsillar pillars, the glossotonsillar sulci, the pharyngeal tonsils, and the lateral and posterior pharyngeal walls (AHNS, 2014). The exclusion criteria included distant metastatic spread, palliative care, second primary tumour and recurrence disease. Additionally, patients were excluded if they were completely edentulous. Further patients

were excluded if their scans had extensive dental artefact leading to poor visualisation of the interested regions and did not allow for accurate dental contouring. Following these exclusions, a total of 160 OPC patients were available. Clinical information was also collected for each patient, which included basic demographics (gender, age at time of diagnosis) as well as oncological details (OPC sub-site, HPV status, tumour size classification, nodal size classification, total Gy).

5.2.3 Radiation treatment

All patients were treated with IMRT. All but 4 patients received 65 Gy in 30 fractions to the tumour site. Of the 4 patients who did not fall into this cohort, 2 received 70 Gy in 35 fractions and 2 received 55 Gy in 20 fractions.

5.2.4 Dosimetry

The post-IMRT scans were all duplicated for the purpose of generating new isodose contours. The Monaco® Treatment Planning System was used for contouring of all dental organs. Contouring commenced at the cementoenamel junction of the tooth and included bucco-lingually bone in a vertical dimension (Figure 5.1). This contour was maintained axially encompassing the roots and therefore the tooth socket as well as the surrounding dento-alveolar bone. Each site was contoured to two CT slices beyond the apex of the teeth in the maxilla and to the level of the mental foramen in the mandible (Figure 5.2). Each CT slice represents 1.25mm. Only the bony components and dentinal roots were contoured. All other anatomy such as maxillary sinuses were not included in the contour. The contoured teeth were labelled according to anatomical position (upper or lower jaw, left or right side and 1 to 8 as per the routine dental notation). Hence a lower right canine was denoted as LR3. A maximum of 32 contours were outlined per patient using the standardised notation. Where multiple teeth were missing a minimum of one tooth per sextant was required to allow for dental contours to be outlined accurately. Edentulous jaws were excluded due to lack of guidance for contouring.

Figure 5.1: Axial slice one of the patient contours of the mandible with the structure denoted in the right column

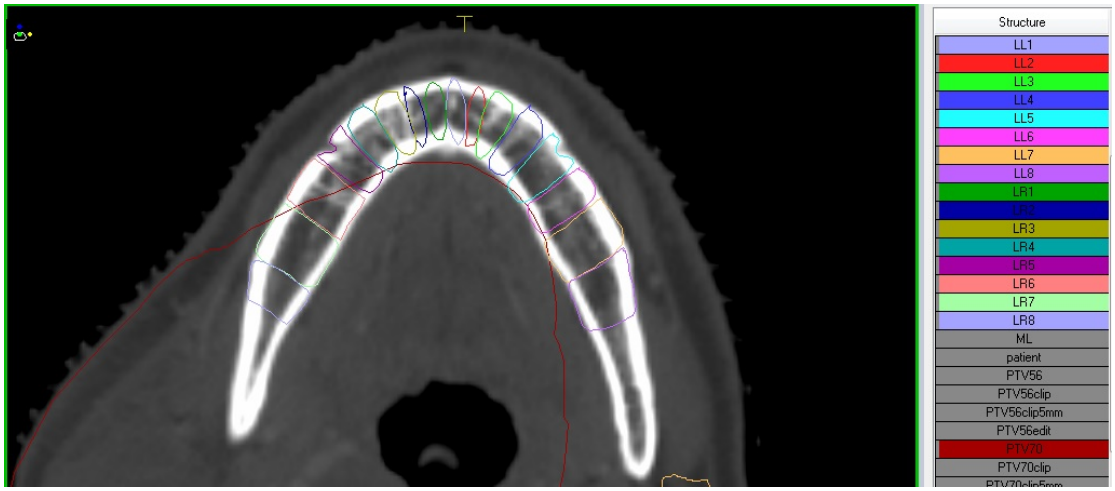


Figure 5.2: Axial slice of same patient with dental contours at the level of the mental foramen

5.2.5 Validation

This was a single observer (VP) study completing the contouring of all the patients. VP repeated contouring on one of the scan 10 times to test the reproducibility and reliability of the contouring technique. A total of 16 (10%) of scans were randomly audited by a consultant oncologist to check for accuracy of contouring and dose retrieval.

5.3 Statistical analysis

5.3.1. Tooth level

Dose-volume histogram (DVH) data was exported per tooth per patient and the mean dose (Dmean), maximum dose (Dmax) and minimum dose (Dmin) calculated using the DVH metrics package (R software). Average doses of the Dmean, Dmax and Dmin were then calculated for all 160 patients and computed further on the basis of various explanatory attributes (tumour size, nodal size, gender, tumour laterality and OP sub-site).

5.3.2 Quadrant level

Using a linear regression model, the average Dmean and Dmax were calculated by splitting the jaws symmetrically via the midline to create 4 quadrants; lower left quadrant (LLQ), lower right quadrant (LRQ), upper left quadrant (ULQ) and upper right quadrant (URQ). The 4 quadrants included the Dmean and Dmax of 8 tooth sites (3 molars, 2 premolars, 1 canine, 2 incisors). Values calculated for each quadrant were used as target attributes in a linear model using the tumour size, nodal size, gender, laterality and OPC sub-site as explanatory attributes. The baseline model (intercept) was set as T1, N0 for a male patient with midline OP (other) tumour.

5.4 Results

A total of 160 OPC patients were included within this cohort study. Figure 5.3 shows the demographic breakdown of the patients. Males heavily dominated the group (n= 121, 76%). There were a similar number of left and right-sided tumours (44% and 41% respectively) with tonsillar tumour (n=83, 52%) being the most common sub-site. A total of 136 patients underwent chemo-RT with the remaining 24 having RT only.

Figure 5.4 shows the overall range of tumour severity based on tumour size and nodal status. T2 size tumors and N2 involvement were the most populated sub-group individually and jointly reflecting the presentation seen in clinical practice.

5.4.1 Tooth level

Observationally for the total cohort, the average Dmean, Dmax and Dmin of a specific tooth was near identical regardless of sides when purposefully excluding tumour laterality (Figure 5.5). Considering tumour side, the data is re-presented in Figure 5.6 and

shows average Dmean doses observed in the anterior jaws appear similar regardless of tumour side. However, in the molar dentition a distinct dose difference is evident in both jaws for Dmean, Dmax and Dmin based on tumour laterality. Higher dental doses were evident on the ipsilateral tumour site compared to the contralateral tumour side.

Respecting tumour laterality the average Dmean doses were subsequently re-tabulated using common explanatory attributes of gender (Figure 5.7), OPC sub-site (Figure 5.8), tumour size (Figure 5.9) and nodal status (Figure 5.10). Minimal differences in average Dmean dental doses were seen between genders. For tumour sub-sites, BOT and OPC (other) were seen to have higher mandibular average Dmean doses than tonsillar tumours. In both tumour and nodal status, a similar observational trend was detected in the mandible and maxilla. With increase grading average Dmean dental doses remained similar in the posterior region on the ipsilateral side. However, the contralateral side dental doses increased with enlarging tumour and nodal status. In the anterior region, minimal differences in dental doses were seen regardless of laterality.

Dmean individual tooth doses for each TNM stage are presented in the supplementary section (Supplementary figure 1-8). Additionally, the data from this study has been adapted to allow for appropriate comparison to other dental dosimetry studies also presented in the supplementary section (Supplementary figure 9-12).

Demographic		Number
Gender	Male	121
	Female	39
Sub-site	Base of tongue	53
	Tonsil	83
	Oropharynx (other)	24
Side	Left	71
	Midline	24
	Right	65
Oncology Treatment	Radiotherapy	24
	Chemo-radiotherapy	136
Total		160

Figure 5.3: Demographic split for all 160 OPC patients

	T1	T2	T3	T4	Total
N0	4 (1/2/1)	13 (5/4/4)	6 (0/3/3)	7 (1/0/6)	**30 (7/9/14)**
N1	4 (2/0/2)	5 (3/0/2)	3 (2/0/1)	2 (0/1/1)	**14 (7/1/6)**
N2	22 (11/3/8)	41 (24/3/14)	22 (7/4/11)	22 (4/3/15)	**107 (46/13/48)**
N3	1 (1/0/0)	3 (2/1/0)	2 (1/0/1)	3 (1/0/2)	**9 (5/1/3)**
Total	**31 (15/5/11)**	**62 (34/8/20)**	**33 (10/7/16)**	**34 (6/4/24)**	**160**

Figure 5.4: Cohort divided based on tumour and nodal status. Laterality is present in brackets as (right/midline/left)

N = 160		UR8	UR7	UR6	UR5	UR4	UR3	UR2	UR1		UL1	UL2	UL3	UL4	UL5	UL6	UL7	UL8
	DMax	62.7 (9.4)	61.0 (10.2)	57.7 (11.0)	51.8 (10.6)	46.8 (10.7)	42.8 (10.2)	39.8 (9.6)	38.5 (9.4)		38.4 (9.6)	39.7 (10.2)	42.2 (11.1)	46.4 (11.6)	51.0 (11.8)	57.8 (11.4)	61.6 (9.9)	63.6 (8.4)
	DMean	56.4 (11.1)	52.4 (11.3)	48.4 (11.3)	44.8 (10.4)	40.0 (10.6)	36.1 (10.0)	34.3 (9.3)	33.0 (8.9)		33.2 (9.1)	34.6 (9.9)	36.3 (10.8)	39.8 (11.3)	43.8 (11.5)	48.6 (11.3)	53.2 (11.3)	57.5 (10.6)
	DMin	46.5 (12.9)	40.3 (12.3)	37.0 (11.7)	36.1 (11.0)	31.9 (11.2)	28.2 (10.5)	28.1 (9.7)	26.8 (9.2)		27.1 (9.3)	28.9 (10.3)	29.4 (11.2)	32.4 (11.8)	35.3 (11.9)	37.1 (11.0)	40.7 (12.0)	47.8 (12.7)
	DMin	48.3 (11.2)	46.7 (10.7)	44.1 (10.0)	42.2 (9.4)	38.5 (8.8)	35.3 (7.9)	33.8 (7.5)	32.9 (7.5)		32.7 (7.6)	33.3 (7.9)	34.3 (8.3)	37.9 (9.4)	42.0 (10.1)	44.0 (10.3)	46.1 (10.9)	48.0 (10.8)
	DMean	56.5 (10.0)	55.6 (9.9)	53.1 (9.6)	49.0 (9.6)	44.9 (9.1)	41.5 (8.4)	39.0 (8.0)	38.0 (8.0)		37.8 (8.0)	38.4 (8.2)	40.2 (8.8)	44.3 (9.8)	49.0 (10.2)	52.9 (9.9)	55.5 (9.8)	56.5 (9.8)
	DMax	63.0 (8.6)	62.6 (8.6)	60.6 (9.0)	55.7 (9.5)	51.3 (9.3)	47.8 (8.8)	44.6 (8.6)	43.6 (8.5)		43.3 (8.7)	44.0 (8.9)	46.5 (9.2)	50.9 (10.0)	55.7 (10.1)	60.7 (9.2)	62.9 (8.6)	63.1 (8.5)
		LR8	LR7	LR6	LR5	LR4	LR3	LR2	LR1		LL1	LL2	LL3	LL4	LL5	LL6	LL7	LL8

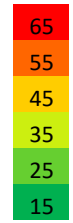


Figure 5.5: Average radiotherapy doses per dental organ with standard deviation as DMax, DMean and DMin of all 160 OPC patients regardless of any specific explanatory attributes or laterality.

	Tumour laterality		UR8	UR7	UR6	UR5	UR4	UR3	UR2	UR1		UL1	UL2	UL3	UL4	UL5	UL6	UL7	UL8
	Left	DMax	60.4	58.7	56.2	50.3	46.6	42.1	39	38		38.5	39.9	42.4	46.7	51.9	60.5	65.1	66.8
		DMean	52.3	49.1	46.8	43.1	39.1	34.6	33	32.2		33.4	35.5	37.2	40.4	44.5	50.6	57.4	62.2
		DMin	41.2	37.1	35	33.9	30.3	26.3	26.4	25.9		27.3	30	30.7	33.7	36.4	39.1	43.9	53.4
	Midline	DMax	61.5	58.5	54.9	49.9	44.7	40.8	38.5	38.1		37.5	39.1	41.6	45.6	50.2	57.6	61.6	64.4
		DMean	56.1	50.7	46.2	43.5	37.6	34.6	33.2	31.9		31.9	33.6	35.3	38.5	42.4	47.8	52.5	57.8
		DMin	47.2	39.5	35	35.9	29.9	27.2	27.5	25.2		25.6	27.8	28.2	31.2	33.6	37.2	40.3	48
	Right	DMax	65.8	64.5	60.3	54.1	47.9	44.4	41.1	39.2		38.6	39.7	42.3	46.3	50.3	54.9	57.5	59.7
		DMean	61.2	56.7	51	47.2	41.9	38.3	36	34.2		33.5	34.1	35.8	39.6	43.6	46.6	48.7	52.2
		DMin	52.2	44	39.9	38.7	34.5	30.7	30.3	28.5		27.4	28.1	28.2	31.3	34.7	34.9	37.2	41.4
N = 160																			
	Right	DMax	67.3	66.9	65.1	58.3	52.4	48.4	44.9	43.8		43.4	43.7	45.8	49	52.1	55.8	57.9	58.3
		DMean	63.1	61.5	57.1	50.8	45.9	42.4	39.3	38		37.7	38.1	39.5	43.1	46.8	48.5	49.1	49.5
		DMin	56.4	53.1	47.8	44.1	40.1	36.7	34.3	33		32.6	32.8	33.5	37.1	40.6	40.2	39.5	40.1
	Midline	DMax	61.9	61.4	59.4	54	48.7	46	43.7	43		42.8	43.2	45.4	48.9	53.9	59.3	62.7	63.4
		DMean	55.3	54.7	51.7	47.1	42.5	39.9	38.3	37.8		37.6	37.6	38.9	42.5	46.8	51.1	54.3	56
		DMin	47	46.1	42.4	39.7	36.7	34.3	33.3	33		32.5	32.3	33	36.2	39.5	42	44.2	46.7
	Left	DMax	59.3	59	56.9	54	51.3	47.8	44.6	43.6		43.5	44.4	47.5	53.3	59.5	65.6	67.5	67.5
		DMean	50.8	50.4	49.9	48.1	44.7	41.2	39	38		38.1	40	41.2	45.9	51.9	57.6	61.7	63
		DMin	41.3	41	41.3	41.3	37.7	34.4	33.5	32.7		32.9	34	35.4	39.3	44.3	48.2	52.9	55.7
			LR8	**LR7**	**LR6**	**LR5**	**LR4**	**LR3**	**LR2**	**LR1**		**LL1**	**LL2**	**LL3**	**LL4**	**LL5**	**LL6**	**LL7**	**LL8**

Figure 5.6: Average radiation doses per tooth as DMax, DMean and DMin incorporating only tumour laterality and no other explanatory attributes.

		Tumour laterality	UR8	UR7	UR6	UR5	UR4	UR3	UR2	UR1		UL1	UL2	UL3	UL4	UL5	UL6	UL7	UL8
N = 160	Male (121)	Left	52.5	49.2	46.6	42.8	38.9	34.5	33.2	32.5		33.7	35.6	37.2	40.5	44.7	50.7	57.4	61.9
		Midline	55.1	50.7	46.6	43.9	37.3	34.2	32.9	31.4		31.1	32.9	34.8	38.2	42	47.3	52.3	57.7
		Right	60.7	55.7	49.8	46	40.7	37.2	35.1	33.5		32.5	32.9	34	37.5	41.7	45.3	48	51.4
	Female (39)	Left	51.6	48.5	47.4	44.5	40.2	35	32.4	31		32.5	34.9	36.8	39.9	44.1	50.4	57.5	63
		Midline	62.2	50.8	43.4	41.6	39.5	37	35.3	35.1		36.8	38.4	38.4	40.5	44.9	50.9	54.1	58.6
		Right	62.5	59.1	53.8	50	44.5	40.9	38.2	36		35.7	37	40	44.3	48	49.6	50.4	53.9
	Female (39)	Left	50.5	49.5	50	48.6	45.6	42.1	39.5	38.5		38.8	40.4	43.2	48	53.5	59.3	62.1	62.8
		Midline	56.1	56.9	54.8	49.4	45	42.1	39.6	38.4		37.7	37	38.3	40.9	44.7	50.3	53.1	52.7
		Right	63.4	62.3	58.5	52	46.9	43	39.5	37.6		36.6	37.2	39.4	43.1	45.8	46.7	47.2	47.8
	Male (121)	Left	50.9	50.7	49.8	48	44.5	40.9	38.8	37.9		37.9	38.6	40.7	45.4	51.5	57.2	61.6	63
		Midline	55.1	54.2	51.1	46.6	42.1	39.4	38	37.7		37.5	37.8	39	42.9	47.3	51.3	54.5	56.7
		Right	62.9	61.1	56.5	50.3	45.4	42.1	39.4	38.2		38.2	38.5	39.6	43.1	47.2	49.3	50	50.2
			LR8	LR7	LR6	LR5	LR4	LR3	LR2	LR1		LL1	LL2	LL3	LL4	LL5	LL6	LL7	LL8

Figure 5.7: Average DMean doses per tooth based on gender and tumour laterality.

		Tumour laterality	UR8	UR7	UR6	UR5	UR4	UR3	UR2	UR1		UL1	UL2	UL3	UL4	UL5	UL6	UL7	UL8
N = 160	BoT (53)	Left	52.5	49.2	46.7	43.2	38.9	34.8	33.8	32.6		33.1	34.8	36.5	39.7	43.8	49	55.5	60.8
		Midline	53.5	48.8	45	41.9	35.5	32.3	31.7	30.8		31.2	32.6	33.3	36.6	41.3	46.9	51.9	58.1
		Right	57.8	50.7	44.5	45.3	38.6	34.8	33.6	32.8		32.9	33.3	33.4	36.5	38.7	43.2	47	51.5
	Tonsil (83)	Left	52.3	49.2	47	43.1	39.4	34.4	32.6	32		33.6	35.7	37.2	40.6	44.9	51.8	58.4	62.8
		Right	62.4	58.6	52.7	47.4	42.5	39.2	36.8	34.7		33.7	34.3	36.3	40.2	45	47.7	49.4	52.8
	OPC *other* (24)	Left	51.7	47.8	45.8	43	38.7	34.4	32.4	31.6		34.1	36.9	39.7	42.2	45.8	50.6	59.5	63.8
		Midline	58.6	52.6	47.4	45.2	39.8	37	34.7	33.1		32.5	34.7	37.3	40.4	43.6	48.6	53.1	57.5
		Right	61.1	58.3	55.3	50.3	47.3	41.1	36.6	34.3		33.6	34.5	38.5	42.8	45.3	47.2	48.2	48.3
	OPC *other* (24)	Left	49.7	50.3	49.7	48.1	44.3	40.7	39.4	39.4		39.6	40.1	41.5	45.7	50.4	56.3	61.5	63.3
		Midline	55.6	54.4	50.6	45.2	40.7	37.3	35.1	34.6		34.3	34.3	35.9	40.2	45.1	49.8	52.9	54.8
		Right	63.5	63.9	61.5	57.7	52.1	46.6	42.8	40		38.6	38.6	41.3	45.9	49.4	51.1	51.1	50
	Tonsil (83)	Left	49.5	48.8	48.3	46.3	42.5	38.8	36.6	35.6		35.7	36.7	39	43.6	50.2	56.6	61.3	62.8
		Right	63.2	61.4	56.5	49.8	44.8	41.4	38.2	36.7		36.3	36.5	37.9	41.4	45.4	47.2	47.6	48.2
	BoT (53)	Left	53	52.7	52	50.5	47.7	44.5	42	41		40.9	41.8	44.2	49.2	54.7	59.5	62.2	63.1
		Midline	55	54.9	52.8	49	44.4	42.4	41.4	41.1		40.8	41	41.8	44.9	48.6	52.5	55.7	57.3
		Right	62.5	61.3	57.3	51.7	47	43.9	42.2	41.6		41.9	42.9	44.4	47.5	50.2	52.1	53.4	53.5
			LR8	**LR7**	**LR6**	**LR5**	**LR4**	**LR3**	**LR2**	**LR1**		**LL1**	**LL2**	**LL3**	**LL4**	**LL5**	**LL6**	**LL7**	**LL8**

Figure 5.8: Average DMean doses per tooth based sub-sites of OPC and tumour laterality.

		Tumour laterality	UR8	UR7	UR6	UR5	UR4	UR3	UR2	UR1		UL1	UL2	UL3	UL4	UL5	UL6	UL7	UL8
N = 160	T4 (34)	Left	54.4	51	47.8	43.9	40	35.9	34.2	32.8		35	37.8	39.6	42.7	46.5	51.4	57.5	61.6
		Midline	54.1	50.6	48.5	51.9	42.6	40.1	38.7	37.4		36	36.8	40	44.3	49.3	55.1	59.5	64
		Right	63.6	58.2	53.4	48.6	41.6	39.6	36	34.8		34.2	34.3	34.7	36.2	41.5	45.6	48.7	53.2
	T3 (33)	Left	53.2	48.9	46.7	42.8	38.7	34.3	32	31.1		31.5	32.7	34	37.8	42	50.1	56.3	61.2
		Midline	44.5	38.1	32.4	29.2	27.8	26.7	26.3	25.2		24.9	27.2	27.6	29.1	31.2	33.8	39.2	46.6
		Right	62.5	60.4	56.3	53.4	49.7	44.9	42	39.5		38.2	41.2	45.2	49.4	51.6	52	51.8	54.9
	T2 (62)	Left	53.3	49.7	48	44.4	39.9	35.6	34.1	33.3		34	36	37.7	40.5	44.4	49.9	58	63.6
		Midline	64.3	60	55.7	50.6	44	39.4	37.6	35.7		36.4	38.7	40.4	44.1	48.8	53.7	56.6	60.7
		Right	61.7	56.7	50.7	45.9	41.5	38.2	35.9	33.8		33.1	33.4	34.8	38.6	42.2	45.8	48.4	52.3
	T1 (31)	Left	45.4	44.3	42.6	40	36.6	30.6	30.3	30.5		32	33.8	35.6	39.2	44.4	50.9	57.9	62
		Midline	59	51	45.5	44.9	34.6	31.4	29.4	29		29.1	29.8	32	35.7	39.7	49.6	57.1	62.7
		Right	58.5	53.7	47.3	45	37.4	33.8	32.4	31.4		30.9	30.9	32.4	36.6	42	45.3	47.5	49.6
	T1 (31)	Left	41.9	43.5	45.6	44.4	40.8	37.4	36.2	35.7		35.7	36.6	39.2	43.9	51.6	58.5	62.3	63
		Midline	54.1	52.7	47.7	42.8	39.2	36.8	35.9	36.2		35.9	35.1	36.1	39.2	44.7	51.1	54.8	56.1
		Right	62.5	60	53.7	45.9	41	37.5	35.1	33.9		33.6	33.2	34.4	38.5	42.2	44.5	45.7	46
	T2 (62)	Left	48.9	48.6	48.5	46.7	43	39.4	37.7	37.1		37.5	38.5	40.7	45.6	51	56.8	61.4	63.1
		Midline	62.5	61.9	59.7	54.2	47.9	43.2	39.9	38.4		38.1	38.7	40.9	46	50.7	54.5	56.2	57.2
		Right	62.9	61.3	56.6	49.9	45.2	41.4	38.6	37.5		37.4	37.9	39.5	43.2	47	48.9	49.5	50.2
	T3 (33)	Left	52.5	51.5	49.4	47.1	43.9	39.5	36.4	35.2		35.5	36.6	38.7	43.7	50.3	57	61.1	62.2
		Midline	49.2	48.4	45.6	42.1	38.9	37.6	36.2	35.8		35.1	34.7	35.4	38	40.7	44	47.9	50.5
		Right	63.6	62.7	60.9	57.5	53.6	49.3	45.5	43.4		43.1	43.8	45.6	49.5	52.2	52.9	51.4	50.5
	T4 (34)	Left	55.5	54.5	53.3	51.8	48.4	45.5	42.9	41.8		41.3	42	44.1	48.6	54	58.3	62	63.3
		Midline	53	53.5	51.3	46.9	42.4	41.1	41.7	42.1		42.9	43.7	44.3	47.9	52.5	56.8	61.1	63.1
		Right	64.9	64.3	62.2	56.1	49.9	48.3	44.9	42.1		40.5	40.6	42.4	43.2	47.8	49.3	51.8	52.6
			LR8	LR7	LR6	LR5	LR4	LR3	LR2	LR1		LL1	LL2	LL3	LL4	LL5	LL6	LL7	LL8

Figure 5.9: Shows average DMean doses per tooth based tumour size and tumour laterality.

		Tumour laterality	UR8	UR7	UR6	UR5	UR4	UR3	UR2	UR1		UL1	UL2	UL3	UL4	UL5	UL6	UL7	UL8
N = 160	N3 (9)	Left	59	56	50.7	46.6	39.8	35.8	32.2	30.6		32.6	35	38	42	48.9	52.7	60.6	65
		Midline	68.2	67	62.3	55.2	45	36.6	33.3	33.2		34.1	39.2	44.7	45.8	44.9	45.9	51.6	55.8
		Right	64	60.2	55.5	50.9	41.4	41	38.2	36.1		36.2	38.6	41.3	44.7	48.1	49.7	51.5	56.7
	N2 (107)	Left	52.6	49.3	47	43.7	39.9	35.5	34	33.1		34.4	36.6	38.1	40.9	44.7	50.4	57	61.4
		Midline	53.1	47.6	43.5	41.1	34.3	31.4	30.6	29.5		29.2	30.1	31.6	35	39.6	46.6	52.5	58.5
		Right	60.7	56	50.5	47	41.8	38.2	36.2	34.3		33.5	34.5	36.7	40.5	44.2	47.6	49.6	52.8
	N1 (14)	Left	49.2	46.8	47.7	45.3	40.2	33.3	32	31.1		31.1	32.5	35.1	39.4	45.7	53.4	59.3	63.7
		Midline	64.9	64.4	60.5	56.8	54.6	53.1	50.4	49.5		49.5	49.5	53.3	57.3	60.7	63.1	64.2	65.9
		Right	63	57.6	50.8	49	43.5	38.3	36.3	34.9		33.8	32.8	33.3	38.3	45.9	46.7	49	52.6
	N0 (30)	Left	50.9	47.5	44.7	39.1	35.7	31.4	30	29.4		30.8	32.6	34	38.5	42.7	49.6	57.3	63.6
		Midline	57.9	51.6	46.5	43.5	39.6	36.9	35	33.2		33.4	36.2	37.5	40.5	44.1	47.8	51.1	56
		Right	61.4	59.4	52.6	44.2	39.4	36.2	31.7	30.3		29.5	27	24.8	27.4	30	31.5	33.8	39.5
	N0 (30)	Left	47.3	47.4	46.7	45.1	42.5	39.2	36.5	34.4		33.7	34.5	36.8	42	48.9	56.7	61.6	63.1
		Midline	53.9	53	50.5	45.6	40.7	37.4	34.8	33.8		33.5	33.5	35.1	38.5	42.6	47.3	51	53.5
		Right	62.1	60	52.7	42.9	38.6	37.1	35.7	34.5		33.2	32.5	31.6	31.7	33.1	33.7	34	35.2
	N1 (14)	Left	42.4	44.8	48.7	48.8	46.5	42.9	40.6	39.3		39	39.7	41.9	46.5	53.6	59.1	62	62.5
		Midline	63.8	64.1	61.4	56.5	52.3	50.2	49.5	48.9		47.9	47.9	49.2	55.1	60.2	63.3	65.4	65.8
		Right	63.9	62.5	57.3	52.5	47.6	42	39.3	39.5		41.6	41.9	40.5	44	46.9	48.8	49.2	50.8
	N2 (107)	Left	52.4	51.4	50.4	48.6	44.9	41.5	39.5	38.9		39.2	40.2	42.4	47	52.6	57.7	61.6	62.9
		Midline	54.6	54.1	50.6	46.6	42.5	40.6	39.8	39.8		39.5	39.5	40.2	43.5	48	52.3	55.5	56.9
		Right	63.1	61.6	57.6	51.5	46.7	43	39.8	38.2		37.7	38.1	40.2	44.5	48.5	50.5	51.2	51.4
	N3 (9)	Left	59.9	60.1	58.1	53.8	48.4	42.4	38.8	38		38.1	39	41	46	52.1	58.1	62.7	64
		Midline	68.8	67.8	66.4	57.3	49.1	42.8	38.7	37.8		38.7	40.8	45.9	54.6	57	58.2	56.1	57.9
		Right	63.1	61.5	58.2	53.5	46	44.3	41	38.9		38.6	40.3	43.3	45.5	49.6	50.3	51.2	50
			LR8	LR7	LR6	LR5	LR4	LR3	LR2	LR1		LL1	LL2	LL3	LL4	LL5	LL6	LL7	LL8

Figure 5.10: Average DMean doses per tooth based nodal status and tumour laterality.

5.4.2 Quadrant level

Quadrant analysis identified Dmean and Dmax for LLQ and LRQ to be statistically significant ($p < 0.0001$). Analysis of the various explanatory attributes revealed intercept, T2, T4, N2 and N3 to be significant coefficients ($p<0.05$) for both lower quadrants (Dmax & Dmean). Additionally, N1 for the LLQ (Dmax, Dmean) and T3 for the LRQ (Dmean) were also found to be significant ($p<0.05$). Some explanatory attributes were found to be highly significant ($p<0.01$) and have been identified in Figure 5.11. For the upper left quadrant (ULQ) and upper right quadrant (URQ) the Dmean and Dmax were not significant considering the R-squared value.

Figure 5.12 shows the minimum, median, average and maximum Dmean doses of each quadrant for the various explanatory attributes. Box plot analysis identified that Dmax and Dmean consistently showed the same trend for each explanatory attribute for each quadrant. Dmean boxplots are shown in figure 5.13-5.17.

5.4.2.1 Gender

A minimal difference (1.4Gy) was observed between males and females regarding median Dmean doses for all 4 quadrants. In all cases the female group has the highest median Dmean dose whereas males had the maximum Dmean dose. Box plot analysis (Figure 5.13) highlighted the male populations had a much wider range of doses for all 4 quadrants.

5.4.2.2 Laterality

As expected, the ipsilateral lower quadrant had the highest median Dmean followed by midline tumours and then contralateral tumours (Figure 5.14). For the maxilla however, the median Dmean doses for the ipsilateral and midline tumours were very similar with the contralateral side being lower.

5.4.2.3 Sub-site

There appeared to be no consistent identifiable general trend between various OPC sub-sites in the mandible. In the maxilla, however, BOT median Dmean dose was lowest of the 3 sub-sites but had the widest range of doses for all 4 quadrants and also consistently showed to have the maximum Dmean dose (Figure 5.15).

5.4.3.4 Tumour size

Box plot analysis (Figure 5.16) showed a general trend of increasing median Dmean dose with increasing tumour size for the mandibular quadrants. However, in the maxilla the median Dmean dose trend appeared to be relative stable regardless of the changing tumour size.

5.4.3.5 Nodal involvement

Box plot analysis (Figure 5.17) highlighted an overall upward trend of the median Dmean dose with increasing nodal classification for all quadrants. The increasing difference between N0 and N3 for the lower jaw was approximately 5.1Gy while for the upper jaw was 6.5Gy. Most interesting was the maximum Dmean dose was consistently seen in N2 for all 4 quadrants (62.6 – 65.9 Gy). N2 also consistently showed to have a wide range on box plot analysis.

Target Attribute	Overall p value	Adjusted R-squared value	Explanatory Attribute	p value
LLQ Dmax	<0.0001*	0.2570	Intercept	<0.0001 #
			T2	0.0091 #
			T4	0.0081 #
			N1	0.0046 #
			N2	0.0001 #
			N3	0.0032 #
LLQ Dmean	<0.0001*	0.2450	Intercept	<0.0001 #
			T2	0.0219
			T4	0.0116
			N1	0.0028 #
			N2	<0.0001 #
			N3	0.0174
LRQ Dmax	<0.0001*	0.1759	Intercept	<0.0001 #
			T2	0.0105
			T4	0.0003 #
			N2	0.0125
			N3	0.0123
LRQ Dmean	<0.0001*	0.191	Intercept	<0.0001 #
			T2	0.0123
			T3	0.0194
			T4	0.0001 #
			N2	0.0070 #
			N3	0.0414
ULQ Dmax	0.3842	0.0060	Intercept	<0.0001 #
ULQ Dmean	0.2797	0.0169	Intercept	<0.0001 #
URQ Dmax	0.1848	0.0305	Intercept	<0.0001 #
URQ Dmean	0.0465	0.0667	Intercept	<0.0001 #

Figure 5.11: Mean dose of each dental quadrants split for Dmean and Dmax for the overall OPC cohort. *p value < 0.05 denoted with * with adjusted R square values >0.1.* Each quadrant is then further split to show those explanatory attributes that were statistically significant *(p<0.05 with p<0.01 denoted with #)*

Attribute	Explanatory Attribute	Minimum (Gy)	Median (Gy)	Average (Gy)	Maximum (Gy)
LLQ Dmean	Male	24.48	47.64	47.01	65.07
	Female	21.93	48.60	46.22	58.98
	Midline	24.58	45.61	51.13	54.35
	Left	37.47	49.27	49.84	64.86
	Right	21.93	45.21	43.91	65.07
	OP - other	24.58	45.84	45.76	61.85
	Tonsil	21.93	46.12	45.01	61.24
	BoT	29.08	50.11	50.02	65.07
	T1	21.93	45.06	43.52	58.98
	T2	24.09	46.78	46.33	61.24
	T3	24.48	48.42	46.72	61.56
	T4	27.95	50.88	50.93	65.07
	N0	21.93	43.70	42.31	55.96
	N1	28.92	48.31	48.45	60.93
	N2	24.48	48.18	47.78	65.07
	N3	41.63	48.71	48.11	57.90
LRQ Dmean	Male	23.67	47.47	46.78	65.92
	Female	26.11	48.44	48.27	60.58
	Midline	23.67	48.68	45.91	55.84
	Left	26.11	45.80	45.27	63.26
	Right	35.90	49.52	49.68	65.92
	OP - other	23.67	47.13	46.41	56.68
	Tonsil	26.11	47.23	46.37	62.30
	BoT	28.84	48.78	48.65	65.92
	T1	31.49	42.87	43.60	53.13
	T2	26.11	48.78	47.66	58.30
	T3	23.67	47.32	46.94	62.82
	T4	28.84	50.55	49.73	65.92
	N0	23.67	45.14	43.50	53.80
	N1	31.49	45.80	48.19	62.82
	N2	28.84	48.58	47.84	65.92
	N3	45.02	50.19	50.17	54.99

Figure 5.12a: Minimum, median, average and maximum Dmean doses for each mandibular quadrant for various explanatory attributes.

Attribute	Explanatory Attribute	Minimum (Gy)	Median (Gy)	Average (Gy)	Maximum (Gy)
ULQ Dmean	Male	3.178	44.75	42.89	64.09
	Female	24.84	45.93	44.85	57.04
	Midline	5.20	44.42	42.48	59.58
	Left	27.95	45.93	45.06	61.52
	Right	3.18	43.61	41.66	64.09
	OP - other	26.18	43.99	44.04	61.52
	Tonsil	20.31	45.43	43.86	59.21
	BoT	3.18	44.63	42.31	64.09
	T1	3.18	44.33	41.86	56.68
	T2	20.31	44.94	43.41	59.58
	T3	5.20	44.75	42.25	55.88
	T4	27.95	45.60	45.54	64.09
	N0	20.31	41.88	40.97	59.58
	N1	23.03	47.11	45.45	57.94
	N2	3.178	45.23	43.53	64.09
	N3	29.83	48.33	45.34	55.70
URQ Dmean	Male	2.903	44.60	43.03	62.61
	Female	21.55	46.02	44.96	57.54
	Midline	2.90	46.55	43.23	60.25
	Left	19.02	41.67	41.10	55.45
	Right	29.38	47.66	46.49	62.61
	OP - other	27.68	46.21	44.52	60.25
	Tonsil	19.02	45.53	43.95	57.49
	BoT	2.90	42.76	42.35	62.61
	T1	27.88	42.94	41.68	53.04
	T2	21.55	46.04	45.00	60.25
	T3	2.90	42.36	41.79	60.41
	T4	32.16	42.90	43.70	62.61
	N0	21.55	40.89	40.42	60.25
	N1	32.30	44.76	45.76	60.41
	N2	2.90	45.47	43.75	62.61
	N3	37.94	47.43	45.95	51.57

Figure 5.12b: Minimum, median, average and maximum Dmean doses for each maxillary quadrant for various explanatory attributes.

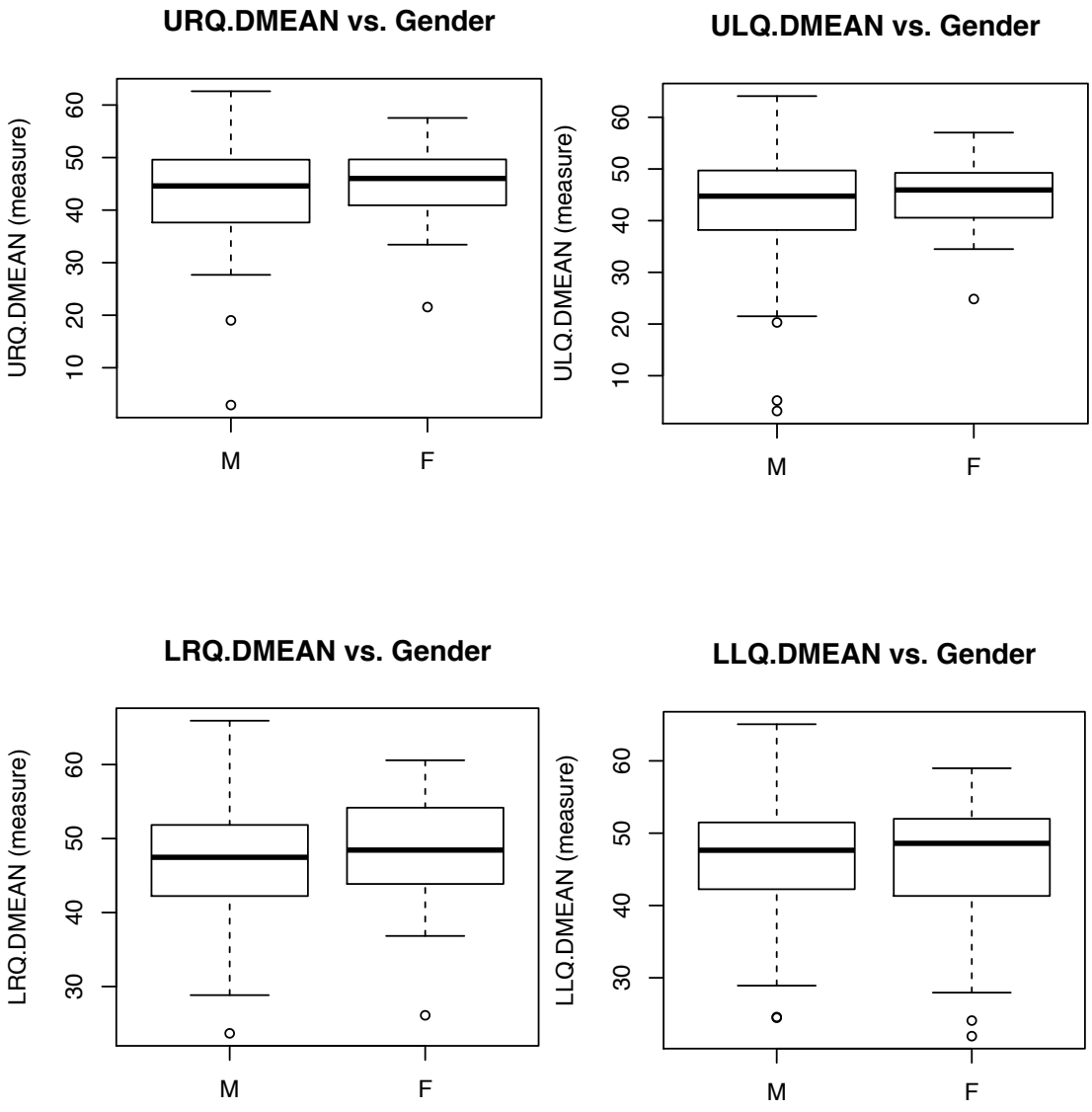


Figure 5.13: Box plots showing Dmean doses for each quadrant based on gender

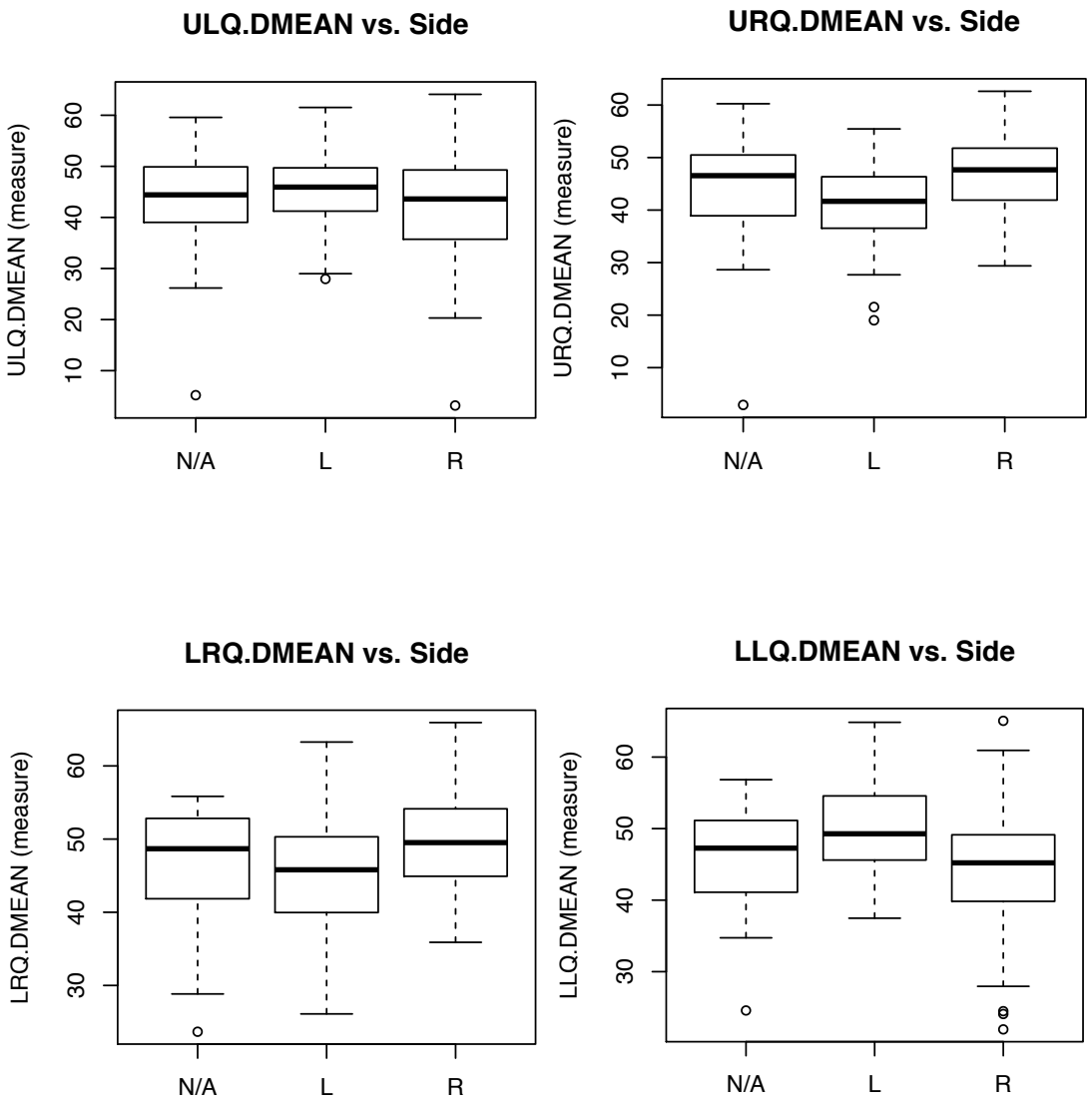


Figure 5.14: Box plots showing Dmean doses for each quadrant based on tumour laterality

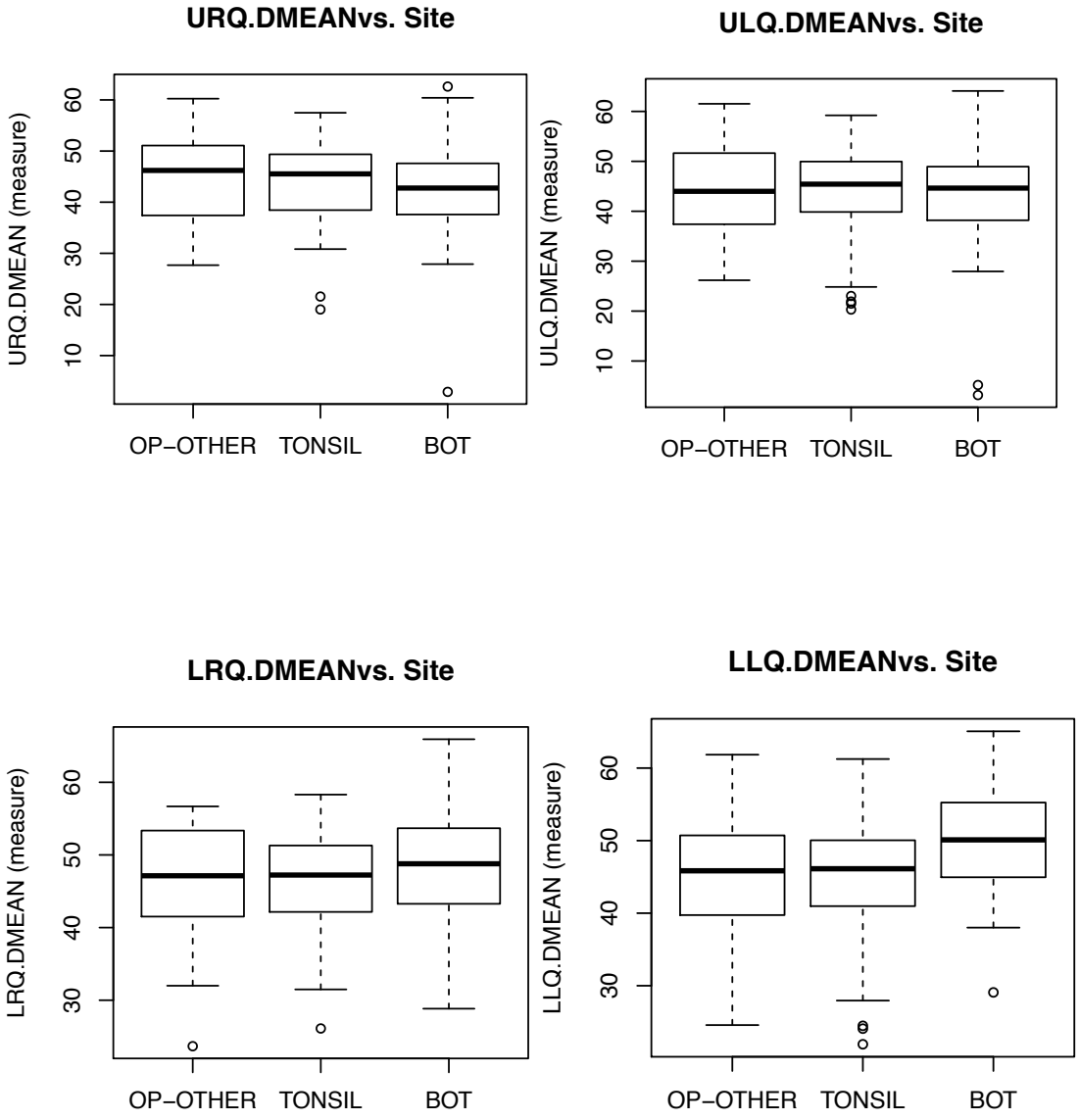


Figure 5.15: Box plots showing Dmean doses for each quadrant based on OPC sub-site

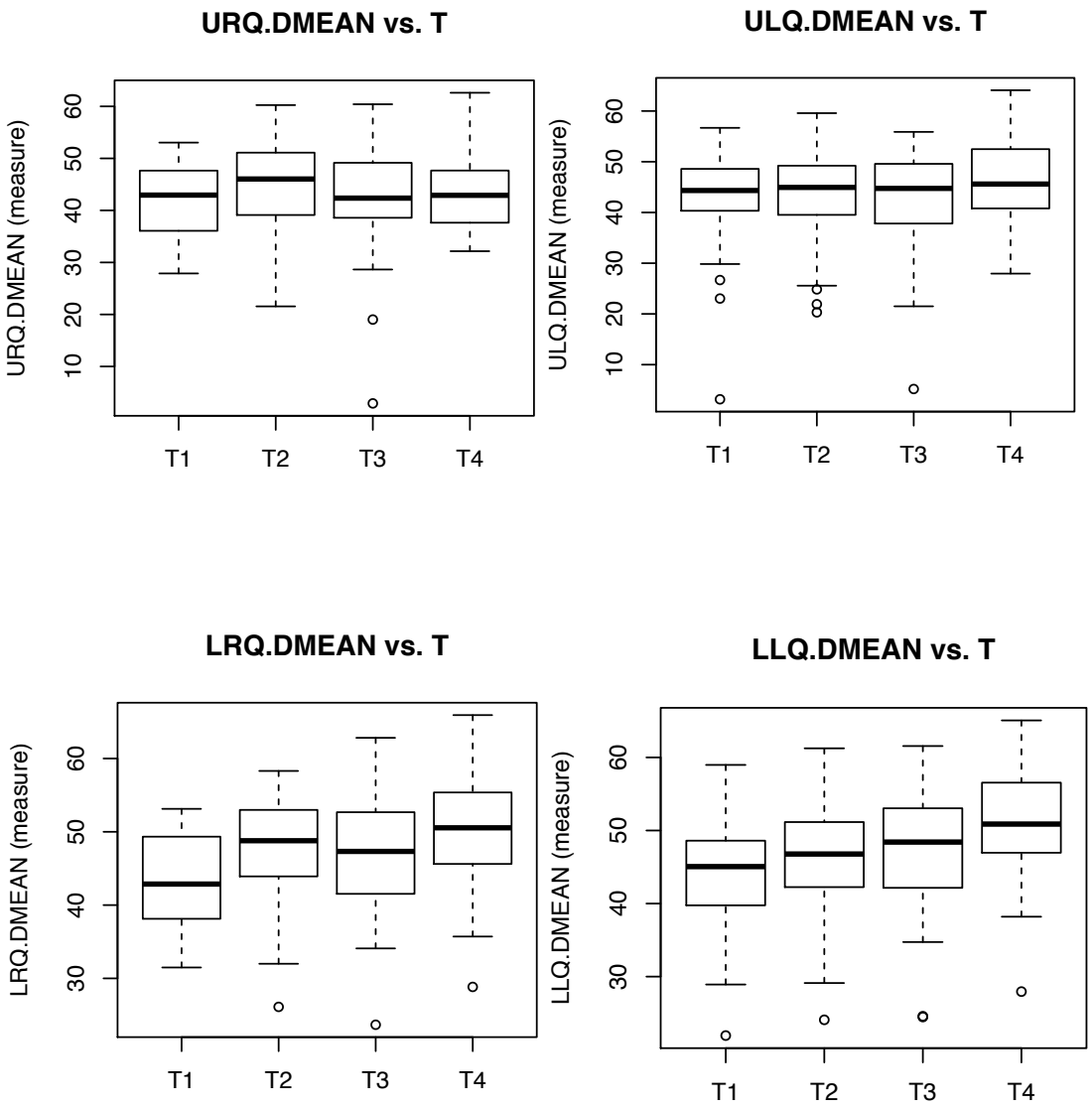


Figure 5.16: Box plots showing Dmean doses for each quadrant based on tumour size (T)

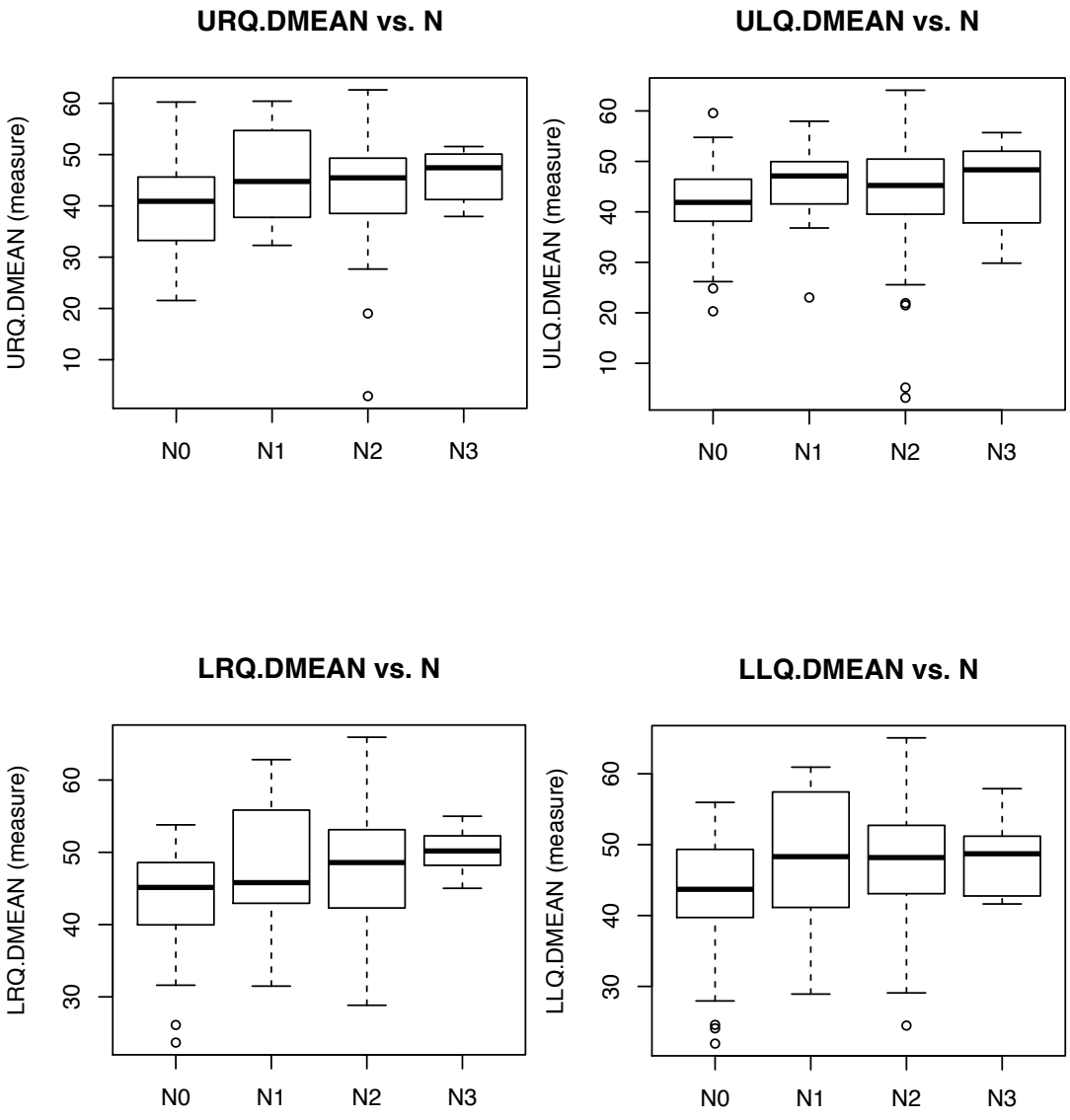


Figure 5.17: Box plots showing Dmean doses for each quadrant based on nodal classification (N)

5.5 Discussion

Radiation dose is considered the most significant predictor for ORN (Ben-David et al., 2007) and studies routinely report a relationship of maximum RT doses >60–75Gy (Ben-David et al., 2007; Chen et al., 2016; Glanzmann & Grätz, 1995; A. Owosho et al., 2017; J. J. Thorn et al., 2000), however doses exceeding 40Gy place patients 'at risk' of ORN (Rosenthal et al., 2008). There is no international agreement that recognises the critical RT dose which leads to ORN.

Doses to the jaws vary based on HNC sub-sites. It has been reported tooth-bearing areas in LC cases received less than 25Gy while the maxillary and mandibular molar regions for OPC and hypopharynx cancers received 50Gy or higher (Bak et al., 2016). These dental doses in the OPC group when taken into consideration with a further 30 years or more of life may explain the rise of ORN in this cohort (MDAndersonHNCSWG, 2017).

Schoen et al reported impairment in the capacity of bone regeneration with radiation doses higher than 40 Gy (Schoen et al., 2007). Decrease vascularity has also been noted and this together with the bony changes reduces the healing capacity (Poort et al., 2017) and subsequently increases the risk of ORN. There is a direct relation between increasing RT dose and chronic hypoxia of the mandibular bone (Poort et al., 2017). The higher radiation dose leads to increased amount of fibrosis, resorption, lacunae and woven bone while bone-remodelling rates decrease (Poort et al., 2017). In the current OPC cohort using either patient or tumour demographics the median in all quadrants had a Dmean of > 40Gy (Figure 5.12-5.17). Using the 40Gy threshold, the present investigation shows that in OPC patients all quadrants of the mouth are potentially at risk of developing ORN irrespective of additional adverse demographics factors.

The tissue impact of RT >40Gy is not solely on the bone but also the teeth. In doses exceeding 30-35Gy the dental pulp has less sensitivity (Garg et al., 2015). Those teeth receiving 30-60Gy are more susceptible to physical injury (Walker et al., 2011) and microhardness reduces at the cervical enamel junction (de Barros da Cunha et al., 2017) which is likely to contribute to radiation induced caries. Normally one would expect such decay to be painful however it is uncommon for these patients to report pain or sensitivity even with multiple teeth involved. In the current study, RT dental doses >30Gy were consistently seen across the complete dentition. This new finding illustrates that the complete dentition is vulnerable to dental disease and the subsequent development of ORN.

The radiation dose map covers the whole mouth (Figure 5.12-5.17) but highlights the importance of laterality in dental assessment and treatment planning. The dose where laterality was disregarded (Figure 5.5) compared to where it was incorporated (Figure 5.6) shows important differences.

Doses outlined in Figure 5.6 and supplementary figures 1-8 highlights the need to re-evaluate the practice of prophylactic molar extraction of sound teeth, particularly on the contralateral aspect. In ipsilateral tumours, regardless of tumour and nodal status, mandibular third molars and second molars receive on average >60Gy. Previous studies assessing dental dosimetry in OPC patients (Bak et al., 2016; Hansen et al., 2012; Parahyba et al., 2016; Rosenthal et al., 2008) have consistently shown doses in the posterior jaw above 40Gy. These reported doses may explain why pre-RT dental extractions is not always successful in avoiding ORN when considered alongside the delayed bone physiology turnover in RT areas.

More concerning is the dental doses to the anterior jaws; Dmean 31.9 – 42.4Gy (Figure 5.6). This finding is consistent with the doses reported by Rosenthal et al showing a significantly larger part of the anterior mandible may be subjected to a ‘‘beam path” toxicity unobserved in previous eras (Rosenthal et al., 2008). Paradoxically clinical experience shows the anterior mandible is a safe area of the mouth. Though the current study did not contour the submandibular gland specifically, anatomically the gland sits in close proximity to the inner aspect of the mandible. Considering both lower quadrants regardless of the attributes assessed were found to have a median and average Dmean dose >40Gy (Figure 5.12-5.17) the submandibular gland will have been exposed to RT. Murdoch-Kinch et al showed the critical dose to the gland to be 39Gy with doses above leading no functional recovery (Murdoch-Kinch et al., 2008). Hence, the secondary impact of this on the dentition is radiation caries which will expedite dental extractions and invite the risk of ORN at these sites.

RT doses in established ORN in OPC has not followed the accepted trend of > 60Gy. Owosho AA et al showed a Dmax range from 44.3-80.9 Gy (average, 69.6Gy) and a Dmean 28.2-74.6 Gy (average, 57.4 Gy) (A. Owosho et al., 2017). However, it needs to be recognised that the state of the dentition also varies (Vinod. Patel et al., 2020; V. Patel et al., 2020) independent of the radiation dose. The OPC group, and particularly the HPV

positive group, present with a better and more complex dentition. This coupled with mean doses >40 Gy predicts for increased dental events and extractions over time (Gomez et al., 2011). With all quadrants on average receiving in excess of 40 Gy (Figure 5.12-5.17), paradoxically pre-RT extractions can increase the risk of ORN (N. M. Beech et al., 2017; Tsai et al., 2013) presumably because healing is not complete at the start of radiation.

The role of patient and oncological demographics in RT doses to teeth is poorly understood. These factors can often be used in pre-RT dental treatment planning either consciously or even subconsciously based on anecdotal evidence or clinician experience. Without any demographic input including the exclusion of tumour laterality (Figure 5.5) the RT doses to the teeth on mandible and maxilla on the left and right appear essentially identical. Introduction of tumour lateralisation (Figure 5.6) highlights that the contralateral dentition receives less dental RT doses compared to ipsilateral side. Blanket molar extraction pre-RT involving the contralateral side is ill-judged and as discussed in the previous chapter will produce a below par functioning patient post-RT.

5.5.1 Gender

Gender was explored for dental dose difference, however, when compared, a minimal difference of 2-4 Gy was observed (Figure 5.12-5.13). Clinically this seems negligible and may lead to disregarding this as a factor. Though their RT doses may be similar their dentitions differ, and both need to be considered jointly. Results presented in the preceding 2 chapters discovered that at the pre-RT dental assessment phase, male patients are reported to have both more teeth and of complex status compared to females (Vinod. Patel et al., 2020; V. Patel et al., 2020) (Chapter4). Add to this the rise of OPC predominantly affecting the male population (Chaturvedi et al., 2013) it is unsurprising that the rate of ORN has increased in this tumour group. For the female OPC population pre-RT dental extractions have been identified as an influential factor towards reduced QoL (N. Beech et al., 2016). Hence, in both genders, dental assessment requires RT doses superimposed over the presenting dentition to provide an individualized treatment plan. Gender has not been consistently reported to be an influential risk factor for ORN. Though ORN is heavily weighted towards males this imbalance is likely to reflect the higher incidence of HNC in this population including in OPC. However, in this study, males were consistently identified as having a higher maximum Dmean doses. Taking this into consideration plus male OPC patients to have a complex dentition and being a populous

group, it is unsurprising that ORN is increasing as these patients survive their tumour diagnosis.

5.5.2 Tumour size and sub-site

Increasing tumour size and nodal involvement inadvertently leads to a wider RT target and subsequent contour. Hence it is naturally assumed this will encompass a wider range of anatomical structures, a conclusion proposed by Hansen et al (Hansen et al., 2012). However, the current study identified the ipsilateral mandibular molars received similar RT doses regardless of tumour and nodal status. The clinical importance of these findings highlights that not all posterior teeth equally receive the same RT doses and therefore blanket extraction policy of these teeth is not necessary. T4 tumours consistently showed the anterior mandible had >40Gy with the remaining tumour and nodal sizes receiving doses between 30-40Gy.

Established tumours that can be consigned to a side are expected to have higher individual RT doses at the tooth level compared to the contralateral aspect. However, on a wider perspective this finding can be argued as misleading. Considering RT doses at the quadrant level, the median Dmean dose difference on the contralateral mandibular quadrant is approximately 3.7-4Gy and for the maxilla 2.3-6Gy less than the ipsilateral side. These doses are only marginally less and clinically could be argued as negligible. At the quadrant level, the mandibular median Dmean and the average Dmean (Figure 5.11) were consistently above the 40Gy ORN threshold regardless of any demographic attribute including laterality. These findings remain a concern considering Abdallah SR et al reported ORN at Dmean of 48.1Gy with the contralateral Dmean only 4.5Gy less which are within the doses identified in this cohort of OPC patients (MDAndersonHNCSWG, 2017).

Mandibular quadrants also showed significantly higher doses compared to the base line model. Specifically, T2 and nodal involvement (Figure 5.12) were particular attributes highlighted. Clinically, OPC patients often first present with larger tumours or nodal involvement. Considering the spatial limitation of the oropharynx region the technique of IMRT with multiple beams mean large sections of the jaws are exposed to RT in order to target the primary tumour even in a lateralised tumour. It is these same spatial limitations that explain why quadrant doses were similar regardless of OPC sub-site (Figure 5.8). Following tumour outline (CTV - clinical target volume) a 1cm safety margin is circumferentially added and therefore most of the oropharynx is likely to be included in

the RT field. Hence, sub-sites may play a marginal difference (Figure 5.15).

Tumour classification in OPC is a recognised risk factor for ORN (Caparrotti et al., 2017) with the current study able to provide supplementary findings to explain this phenomenon. Increasing tumour size identified increasing median Dmean dose in the mandible as expected with larger tumours having larger CTV and encompassing safety margin (planned target volume). In such a constricted environment the involvement of adjacent structures is often inevitable such as the jaws and the dentition. While attempting to avoid vital structures such as the parotid gland and the spinal cord this therefore requires delivery through the mandible, which is often unavoidable particularly where the cervical lymph nodes are involved. T4 tumours were seen as significant explanatory attributes for both lower quadrants (Dmax & Dmean, $p<0.0001$). This finding is unsurprising considering that T4 OPC within their definition encroaches on the jaws (medial pterygoid, hard palate or mandible) and will therefore be involved in the CTV. In the current study T4 was identified as consistently having the highest maximum Dmean dose for all quadrants and a median Dmean doses >50Gy in the mandible.

5.5.3 Nodal stage

Increasing nodal classification highlighted a general increasing trend of the median Dmean. Both N1 and N2 were significant ($p<0.01$) explanatory attribute for mandibular quadrants. The maximum Dmean dose was consistently seen in N2 for all quadrants. This pattern is likely to be related to the presentation of OPC patients but also anatomy. OPC patients commonly present with painless neck lumps and the intra-oral symptoms can be non-existent or minor and therefore ignored (Marur & Forastiere, 2008; O'Sullivan et al., 2012). In a review, OPC patients reported that over 66% (478/721) of patients presented with advanced classification (N2 or N3) nodal disease (Ang et al., 2010). The oropharyngeal lymph drainage is commonly to level I, II and III. Level Ib (submental region) is situated on the inner aspect of the mandible whereas Level II is situated on the distal edge of the angle of the mandible while level III approximately 1cm inferiorly to level II. Hence all 3 levels are in close proximity to the mandible and therefore contour with safety margins are inadvertently likely to involve the jaws. In lateralized T1-T2 tumours without nodal involvement, ipsilateral cervical RT is routine. However, these tumours presentations in OPC are in the minority. More common are larger tumours or nodal involvement, which often involves irradiation of the contralateral neck.

5.5.4 Tumour size & nodal status - TNM

Though trends can be identified the dental dose can only be truly assessed when using tumour and nodal classification together for a single tooth. This is evident when reviewing supplementary figures 1-8 which provides dental doses where the tumour size remains static and the nodal classification changes and then vice-versa. Data presented in this format finally provides dental doses based on the exact TNM stage for each individual tooth for OPC. An obvious example is a right sided T1N0 tumour (Supplementary Figure 1) where a lower right third molar exhibits a dose of 64.6Gy while the lower left third molar 26.8 Gy. Hence, the latter carries a very low risk of ORN and may be considered to be retain if of functional value. Compare this to a right sided T1N2 tumour (Supplementary Figure 1) where the lower right third molar still receives > 60Gy but now the contralateral lower left third molar receives almost 50Gy.

Only with this level of detail can the dental oncologist provide a tailored dental assessment and recommend treatment on a more informed basis. The tables presented in the supplementary section provide all the permutation for the various tumour and nodal grades with Dmean dental dosimetry. This information can now be superimposed over the patient presenting dentition. Poor prognosis teeth in high RT region can be considered for removal knowing it is inevitable they will need removal in the future with the added risk of ORN. Equally, unrestored teeth in a highly motivated patient considered to have long term survivorship can be knowingly kept.

5.5.5 Literature review of dental dosimetry

There are a number of studies that have investigated dental doses in OPC with IMRT (Bak et al., 2016; Hansen et al., 2012; Parahyba et al., 2016; Rosenthal et al., 2008). Though the methodology and the data collected vary a degree of comparison to our data is achievable. A study by Rosenthal et al aimed to determine the difference in doses of IMRT versus 3D conformal RT (Rosenthal et al., 2008). Though their aims were not aligned to the current study their published IMRT dental doses allowed for direct comparison (Supplementary Figure 9). The Dmean doses identified by Rosenthal et al were substantially lower than those identified in our study (Rosenthal et al., 2008). This variance is likely due to vastly lower numbers (n=15) and differing case mix. Of the latter, their study was weighted towards T1-T2 tumours while the current study had larger number of T3-T4 tumours. Regardless, based on their primary aim of comparing 2 differing RT delivery systems they

make a concerning conclusion with clear statistical significance that IMRT doses to non-targeted areas are much higher than of 3DCRT. Of specific interest in the dental regions, all areas of the maxilla and mandible except the posterior mandible showed IMRT delivered significant higher doses of radiation compared to 3DCRT. Hence, in line with our findings areas beyond just the posterior mandible do receive >40Gy meaning pre-RT dental assessment needs expanding to include meticulous assessment of all the teeth in both jaws.

In a more focused study towards IMRT dental doses, Bak SY et al assessed 36 OPC (10 tonsil, 26 BoT) patients (Bak et al., 2016). They concluded that dose distribution is dependent on tumour site but also the geographic proximity of the cancer to the dentition. Compared to our data (Supplementary Figure 10) the doses reported by Bak SY et al were less for all the mandibular teeth except the third molar site (Bak et al., 2016). Teeth contoured as groups rather than individually, non-categorizing of tumour status, nodal status, tumour laterality and limited numbers are likely to explain the discrepancy in their reported doses to this study.

Hansen et al did consider tumour size but grouped them together (Hansen et al., 2012). This study also contoured teeth as anteriors, premolars and molars. Comparison of dental doses (Supplementary Figure 11), showed similar dose alignment in the large tumour sizes. However, in the smaller tumour sizes, Hansen et al reported lower doses than we had identified (Hansen et al., 2012). Again, a number of methodological differences may explain the variation in doses. One vital difference is their contouring encompassed the full mandibular height to the lower border rather than restricted to the dento-alveolar segments. Finally, maxillary teeth were not included in their study. However, their conclusion is comparable which stated tumour size being an important factor for dental dose (Hansen et al., 2012). They also concurred with the advice of Rosenthal et al that the whole mandible should be considered for dental assessment rather than just posterior teeth irrespective of the laterality of the tumour (Rosenthal et al., 2008).

Parahyba et al published the most recent study to assess dental doses in OPC (Parahyba et al., 2016). Again, the doses reported (Parahyba et al., 2016) compared to this study were vastly lower (Supplementary Figure 12). As with the other studies, both methodology and demographic distribution were different. Parahyba CJ et al cohort has a near comparable T1 and T2 group (Parahyba et al., 2016) to this study however the T4

group in the current study was much larger. Regarding the nodal staging, only N3 was comparable in our cohort to theirs. Our study had a much higher percentage of N2 and a vastly lower percentage of N1 compared to theirs. In addition, Parahyba CJ et al had equal split of BOT and tonsillar tumours (Parahyba et al., 2016) whereas the current study had a heavy leniency towards tonsillar tumour. Therefore, direct comparison of data has its limitations based on dissimilar patient population. Demographics such as tumour site, size, laterality and nodal staging are significant factors for the actual dose delivered and this was also the conclusion by Parahyba CJ et al (Parahyba et al., 2016).

Though IMRT is the currently the gold standard for RT there is already advancement towards proton beam radiation therapy (PBRT). Owosho et al compared the dosimetric distribution of ipsilateral PBRT to IMRT in the tooth-bearing region of the mandible in patients with HNC and found it to have effective tissue-sparing capability (A. A. Owosho et al., 2016). However, even at this early stage, PBRT has been reported to cause ORN (W. Zhang et al., 2017). Hence, the principle of jointly considering RT doses and dental status still remains key.

5.6 Conclusion

Dental dosimetry in OPC can vary based on tumour laterality as well as series of oncological demographics. Large areas of the dentition and associated dento-alveolar bone are irradiated and would explain why this particular tumour group is recognised to have an elevated ORN risk.

Pre-RT dental assessment of this group is a complex assessment and based on predicting tooth prognosis, balancing risk of ORN and the background of survivorship. The inclusion of dental dosimetry matrix will allow the provision of a more tailored pre-RT treatment plan.

Isolating the various explanatory attributes consistently shown to have maximum Dmean dose and therefore at most risk of ORN identified a profile of males with a right-sided BOT tumour with a T4N2 OPC tumour.

5.7 Limitations

The current study recognizes a number of limitations.

Considering RT doses, firstly, protocols for tumour volumes and contours may differ between clinical units. Secondly, direction of tumour growth may also vary for the same tumour grading. An obvious example would be a T4 tumour, which extends superiorly into the nasopharynx or equally inferiorly towards the hypopharynx. In such an event arguably the RT in the latter would in comparison encompass more mandible whereas the former would involve more maxilla. Thirdly, patients vary anatomically and present with differing jaw dimensions and tooth positioning within them.

Chapter 6

The impact of radiotherapy as a late effect on the microvascular network of oral mucosa in oropharyngeal cancer patients

This chapter is co-authored with Pedro Bastos (PhD thesis: Real time optical vascular imaging for the diagnosis of oral diseases). It has been edited and adapted to reflect the current PhD thesis theme

6.1 Introduction

The preceding studies have identified that OPC patients receive both a significant RT dose to the jaws with a widespread coverage above the 40Gy threshold of developing ORN. Having also established that OPC and particularly the HPV positive group have a superior dentition this vulnerability places them at risk of development of ORN.

The next phase would be to consider how the soft tissue and in particular the gingival tissue and attached mucosa is impacted by the RT doses it receives. Its presence is the only tissue barrier protecting the dento-alveolar bone with the threat of ORN. In addition, the soft tissue margins abut the dentition which provides yet another portal for microbes to penetrate through to access the vulnerable irradiated underlying bone.

With the most recent pathophysiology of RIF proposed by Delanian et al the loss of vascularity in the long term leads to atrophy and in the case of the jaws, ORN (S Delanian & Lefaix, 2004). RIF leading to atrophy have been shown in breast, skin, liver and kidney and also in the jaws (V. Patel & M. McGurk, 2017). However, in the IMRT era the hypothesis proposed was this will be reduced considering the novel technology is better targeted. However, Rosenthal et al found this was not the case and IMRT showed significant RT doses to non-targeted sites compared to non-IMRT (Rosenthal et al., 2008).

Blood supply to the jaws is predominantly endosseous in early life which then migrates over time to be dominated by periosteum. Hence, the microvasculature of the soft tissue in the adult population receiving HN RT remains vitally important to be able to perfuse the bone. However, HN RT provides a real challenge to the oral cavity with it impacting saliva which has a subsequent impact on the oral microbiome as well as the oral tissues. Lack of perfusion of the vulnerable bone will eventually lead to ORN and based on the RIF and atrophy model proposed by Delanian et al this is likely to progress as time passes following RT (S Delanian & Lefaix, 2004). If this is the case, then in theory OPC patients and in particular HPV positive patients are vulnerable to microvasculature depletion with their long-term survival following RT.

It would be prudent to investigate the gingival and mucosal microvasculature status of OPC patients over time following HN RT. The concern is that if RT does cause depletion of microvasculature of the soft tissue over time then this may provide further insight into why this tumour sub-site have an elevated ORN rate. Assessment of the microvasculature has been attempted via repeat soft tissue biopsies and histopathological analysis (Richter

et al., 2007). The availability of novel and non-invasive imaging techniques such as optical coherence tomography (OCT) and real time optical vascular imaging (RTOVI) has however made the need for invasive investigations redundant. The use of OCT for direct observation of angioarchitecture and morphology in early and late irradiated tissue has been widely assessed (Davoudi et al., 2013; Dekker et al., 2018; Gladkova et al., 2008; Narayan et al., 2008). However, varied results have been reported due to discrepancies. These include either imaging differences of HNC patients, different sites and at differing times. Standardized data acquisition procedures are necessary for providing reliable clinical assessments of irradiation-induced side effects and facilitate identifying correlations associated with different pathologies (e.g mucositis, atrophy, and tissue necrosis) (Helmers et al., 2018).

Having recognized that OPC receive a substantial dose to the teeth and the surrounding soft and hard tissues the long-term impact has not been determined particularly for IMRT. The late impact of RT in microvascular anatomy has not been previously assessed with *in-vivo* microvascular imaging. The current *in vivo* study aims to use RTOVI to acquire site specific microvascular images while simultaneously assessing the exact RT dose to the region to quantify late changes within the keratinised mucosa and gingival tissue of OPC patients previously treated with RT.

6.2 Methodology

6.2.1 Ethics

NRES Ethics 11/LON/0354 and KCL Research Ethics Committee (REC) BDM/14/15-14 approvals.

19/EE/0224 - Dental status, radiotherapy doses and subsequent implications in head and neck cancer patients - A retrospective cohort study

6.2.2 Patient identification

A standardised patient demographic and oncology profile was implemented for this study. Only T2N2 (AHNS, 2014) unilateral biopsy confirmed HPV positive OPC in male

patients treated with IMRT with no history of smoking for a minimum of one year prior to their cancer diagnosis were included.

Eligible patients were identified via the database of patients within the study described in chapter 5. A total of 21 patients were eligible for this study and all patients were invited to participate.

6.2.3 Real time optical vascular imaging

RTOVI is an optical technology developed at GSTFT. The working principle of RTOVI is based on the emission of green light (Wavelength: 520 nm) directed to the examined tissue to benefit from the natural contrast mechanism of haemoglobin inside red blood cells (RBC). Green light is well absorbed by haemoglobin, but the surrounding tissues (e.g. connective tissue) will reflect light back to the examination probe. This allow RTOVI system to obtain a grey scale image defining the trajectory of the RBC which can then be used to describe the microvascular anatomy.

RTOVI is constituted by a light source of a 175 watt Xenon arc lamp (Karl Storz endoscopy – Storz®), filtered by a 520/20 nm band pass filter producing an incoherent monochromatic (green) light, relayed to the tissues via a dedicated fibre-optic cable (Karl Storz® - 495L) and the endoscope's conventional segregated illumination channel (Karl Storz® - Hopkins II 26157RTA). As the green backscattered light is absorbed by haemaglobin pigments, the consequent images show low intensity RBCs moving through the higher intensity background image. Endoscope eyepiece images are relayed via a magnification system (Thorlabs® SM1NR05) to a high resolution (2040 x 2040 pixels) analogue monochrome charge coupled detector (Basler® ACA 4060). A subsequent 8 bit analogue to digital converter (ADC) digitizes the signal (following a binary system), allowing each pixel one of 2^8 (256) different greyscale values. This output is sent to a personal computer where the images are collected and recorded by Basler's Pylon® software. The field of view (FOV) used by RTOVI is 0.72 mm x 0.72 mm.

6.2.3.1 RTOVI technique

All imaging was completed by two experienced operators utilising RTOVI during the period of April – June 2018. Image selection was performed by a single examiner. The first high quality frame after the midframe of each microvascular movie was selected for

analysis. There was an unavoidable subjective input on this step. However, some parameters have been followed as guidance, such as minimal or absence of artefacts and distortion in the FOV (De Backer et al., 2007).

Images were taken from pre-agreed fixed sites of either the gingival tissue or the attached mucosa of the edentulous site following the same image protocol (e.g. magnification, resolution, light intensity). Sites agreed were maxillary and mandibular second molar, second premolar and lateral incisors (UR2, UR5, UR7, UL2, UL5, UL7, LL7, LL5, LL2, LR2, LR5 and LR7) on both the buccal and lingual or palatal aspect for both left and right side.

6.2.3.2 RTOVI analysis

A customized angiogenesis analyzer (CAA) (A. P. Bastos, 2020), a plugin for imageJ, was used to analyze the *in-vivo* images of microvascular anatomy, deriving 20 mathematical parameters (Figure 6.1). CAA was tested and validated in previous work (A. P. Bastos, 2020; P. Bastos & Cook, 2016). A previous RTOVI atlas study comparing a group of 10 healthy volunteers, according to all parameters used by CAA found no difference between the soft tissue microvasculature of the maxilla and mandible (A. P. Bastos, 2020; P. Bastos & Cook, 2016). Additionally, in-depth analysis of the maxilla in the anterior (central incisor region) and the posterior gingival margin (second molar region) also showed no significant difference (A. P. Bastos, 2020; P. Bastos & Cook, 2016). Subsequent analysis assessed the feasibility to differentiate healthy oral cavity tissues from local disease (e.g cancer) which was clearly discernible (A. P. Bastos, 2020; P. Bastos et al., 2017). CAA offers an automated analytical routine based on the microvascular architecture. However, it does not offer image or frame selection (based on image quality) and the removal of artefacts from the FOV. In this instance, image selection and artefacts removal were performed by a single clinical examiner. Subsequently, all results were converted to the same FOV (1 mm^2), allowing a standardized comparison between images. Figure 6.2 shows the image timeline from retrieval to analysis.

Additionally, TIBCO® Spotfire, a visual analytic software, highlighted important discriminatory parameters in the analysis. These parameters were reduced to 4 in order to make feasible the description of the microvascular analysis of this work and simultaneously, transmit the relevant findings. The parameters selected were, the total branches length, number of junctions, number of master segments and outline area.

Parameters	Description of parameters	Unit
Number of extremities	Terminal part of a branch or segment.	Number of objects
Number of junctions*	The link between segments or a branch with a segment.	
Number of master junctions	Junctions connecting segments forming a mesh	
Number of master segments*	Segments forming a mesh	
Total master segments length	The total length in micron of segments forming a mesh	
Number of meshes	Meshes are areas of vascular net. Several segments forming a close circuit. Total Number of meshes.	
Total mesh area	Total area in micron of the sum of all meshes in the FOV	μm^2
Number of pieces	Total number of segments, master segments, junctions, master junctions.	
Number of segments	Segments, different parts (vectors) of the same vascular unit.	
Number of branches	Terminal part of the vascular unit or capillary.	
Number of isolated segments	Segments not connected on both ends.	
Total length	Total length in micron of all segments and branches	μm^2
Total branching length*		
Total segments length	Length in µm of all segments	μm^2
Total branches length	Length in µm of all branches	μm^2
Branching interval		
Mesh index		
Mean mesh size		
Outline area*	Total vascular area	μm^2
Mean Width Index		

Figure 6.1: Mathematical hemodynamic parameters available for assessment via customized Angiogenesis Analyzer (CAA). * identifies the four categories analysed further via TIBCO® Spotfire.

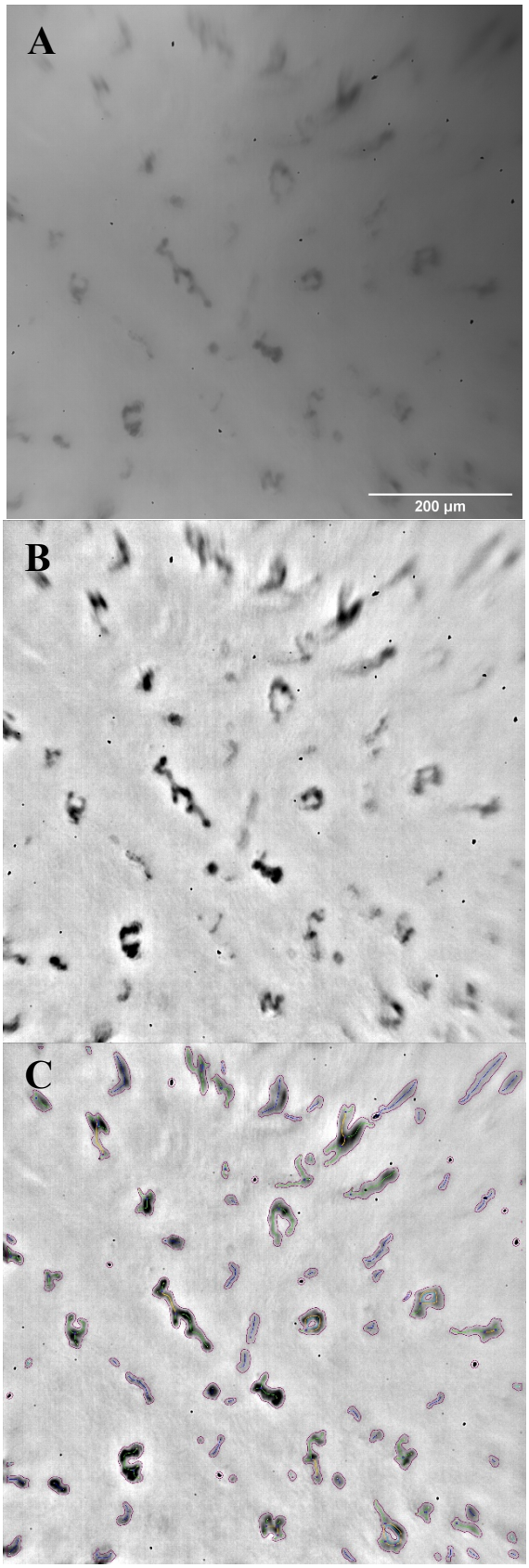


Figure 6.2 : Microvascular image analysis sequence. A) Raw image. B) Image after processing. C) Analyzed imaged.

6.2.4 Dosimetry

Dosimetry for each dental site was available as all patients were sourced from the study described in chapter 5 (Section 5.2.4). Only Dmean and Dmax doses for the maxillary and mandibular second molar, second premolar and lateral incisors for both left and right side were retrieved in line with the regions clinically imaged by RTOVI.

6.3 Statistical analysis

Kolmogorov-Smirnova and Shapiro-Wilk tests demonstrated that the data was not normal distributed. Therefore, non-parametric tests were used for the statistical analysis of this study data. Mann Whitney U test and Kruskal Wallis were used when comparing 2 groups and more than 2 groups, respectively. The level of significance was set at 0.05.

6.4 Results

A total of 13 male participants with HPV positive T2N2 OPC treated with chemo-RT were included in the study. The mean age was 55.8 ± 8.8 years (range 34 – 67 years). Figure 6.3 outlines further presenting demographics. In the control group, 8 healthy and non-smokers male volunteers with a mean age of 67.5 ± 11.8 (range 51 – 76 years) were invited to participate in this study.

The individual dental dose as Dmax and Dmean are presented in Figure 6.4. The average Dmean for the lower second molar, lower second premolar and lower lateral incisor were 56.6 ± 6.8Gy, 50.6 ± 7.9Gy and 38.6 ± 5.0Gy respectively. The figures were marginally lower for the maxilla with average Dmean for the second molar, second premolar and lateral incisor being 53.6 ± 9.7Gy, 45.6 ± 10.8Gy, 36.2 ± 7.9Gy respectively.

Overall, the microvascular parameters within the RT group were decreased when compared to the control group, with 17 parameters identified as significant. p value ranged from 0.001 in the total branch length and 0.054 in the number of master junctions. Within the RT group itself, no significant differences were found according to all 20 microvascular parameters when comparing the upper and lower jaw. However, this was equally true when comparing MVD of the upper and lower jaw in the control group. The p value ranged from 0.143 and 0.952 in the branching interval and number of isolated segments respectively. However, in order to determine if both jaws followed the same pattern of RT related microvascular changes, with the increase of RT dose, both jaws were assessed independently. Similar vascularization patterns were found in the upper and

lower jaws. In the upper jaw there was a decrease in the vascular area of 43.2 % in the posterior region (second molar) and of 22.5 % in the anterior region (lateral incisor) compared to the control group. In the lower jaw, there was a decrease in vascularity of 39.7 % in the posterior region and 22.9 % in the anterior region.

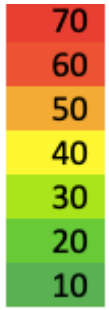
The microvascular gingival anatomy of the ipsilateral cancer side was compared with the contralateral side to establish if any significant difference existed.
In all 21 CAA parameters there was no significant difference (p value = 0.422-0.956) when comparing the ipsilateral side with the contralateral side. To eliminate dilution of the results by including all gingival regions receiving different doses of RT, specifically the second molar region was compared from both sides. As the RT dose map identified this area receives the highest dose. Again, according to all CAA parameters there were no significant differences (p value = 0.179 - 0.904).

Figure 6.5 shows the decreasing value of each category as the time from RT increases when compared to controls. For both categories, this decreasing value was significant compared to controls in both the 2013-14 group (p=0.008 & 0.043) as well as the 2011-12 group (p=0.022 & 0.031). Furthermore, for the outline area comparing the 2013-14 group versus the 2015-16 group the difference was seen as significant (p=0.029).

Analysis of the four key parameters; total branches length, number of junctions, number of master segments and outline area identified a significant ($p \leq 0.001$) reduction on all fronts in the RT group versus the control group. Using these parameters comparing individual dental sites again RT sites had a reduced number in comparison to the control group ($p \leq 0.05$). Data is summarised in figure 6.6. Using box plot analysis (Figure 6.7-6.10), the median value consistently declined as the RT site migrated posteriorly compared to the control group.

Patient Number	Age	Years post-RT	Co-morbidities	Medications	Smoking History	OPC sub-site	Tumour laterality	CRT regime	Chemotherapy medication	Number of infusions
1	49	7	Nil	Nil	Never	Tonsil	Right	Concomitant	Cisplatin	2
2	63	6	Hyperlipidaemia	Simvastatin	Never	Tonsil	Left	Concomitant	Cisplatin	2
3	34	6	Nil	Nil	Never	Tonsil	Right	Induction & concomitant	Cisplatin & 5FU	4
4	59	5	Nil	Sildenafil	Never	Tonsil	Left	Concomitant	Cisplatin	2
5	52	5	Nil	Nil	Ex-smoker*	Tonsil	Right	Induction & concomitant	Cisplatin & 5FU	4
6	48	5	Nil	Nil	Ex-smoker*	Base of tongue	Left	Induction & concomitant	Cisplatin & 5FU	4
7	55	5	Anxiety Depression Acid Reflux	Sertraline Quetiapine Promethazine Omeprazole	Ex-smoker*	Tonsil	Right	Induction & concomitant	Cisplatin & 5FU	4
8	54	4	Type 2 Diabetes Mellitus	Metformin Simvastatin	Never	Tonsil	Right	Induction & concomitant	Cisplatin & 5FU	4
9	58	4	Hypertension Hyperlipidaemia Gout	Ramipril Simvastatin Allipurinol	Ex-smoker*	Tonsil	Right	Concomitant	Cisplatin	2
10	67	4	Hypertension	Indapamide	Ex-smoker*	Tonsil	Right	Induction & concomitant	Cisplatin & 5FU	4
11	63	3	Nil	Nil	Ex-smoker*	Base of tongue	Right	Induction & concomitant	Cisplatin & 5FU	4
12	59	2	Myocardial Infarction Gastric Reflux	Simvastatin Aspirin Omeprazole	Ex-smoker*	Tonsil	Right	Induction & concomitant	Doxcetaxel, Cisplatin & 5FU	5
13	64	2	Nil	Nil	Ex-smoker*	Tonsil	Right	Concomitant	Cisplatin	2

Figure 6.3: Patient cohort with basic demographics. * indicates ex-smokers who had quit over 12 months prior to their cancer diagnosis



Patient		LL7	LL5	LL2	LR2	LR5	LR7	UL7	UL5	UL2	UR2	UR5	UR7
1	*Dmax*	**65.5**	55	43.3	43.8	46.4	**65.7**	**57.7**	45.1	38.1	44.7	48.9	**61.3**
	Dmean	**57**	49.3	39.8	40.1	40.6	**53**	**46.7**	26.9	23.8	38	43.1	**45.2**
2	*Dmax*	**67.2**	68.3	43.1	48.6	63.6	**59.4**	66.2	59.7	46.7	49.6	63.3	65.2
	Dmean	**64.8**	63.8	36.3	41	55.6	**46.8**	62.7	51.9	42.4	43.5	59.7	56.7
3	*Dmax*	60.9	60.7	50.7	46.2	66.7	67.4	61.7	57.7	49.8	46.8	64.4	67.9
	Dmean	52.5	53.3	44.4	40	62.2	64	49.8	51.9	44.9	41.8	57	64.8
4	*Dmax*	**67.6**	60.8	37.4	33.8	58	**68**	67.3	51.5	44.7	41.3	51.9	64.1
	Dmean	**62.8**	55	33.4	31.8	51.6	**62.6**	58.7	46.3	40.4	35.7	44.9	55.8
5	*Dmax*	**68.9**	63.9	51.5	50	60.3	**66.9**	**66.2**	44.7	40.4	40.8	48.3	**64.4**
	Dmean	**59.8**	58.7	39.5	40.5	53.6	**63.3**	**57.1**	41.5	36.5	37.5	42.2	**55.2**
6	*Dmax*	68.2	64.7	49.8	49.7	59.2	65	67.9	60.8	45.3	47.9	52.3	62.1
	Dmean	63.4	59.7	46.1	42.2	52.9	53.9	63.8	52	38.2	43.3	48.8	55.1
7	*Dmax*	**58.4**	**48.4**	**43.1**	**47.3**	**59.1**	**66.1**	**60.8**	**47.1**	**39.3**	**48**	**55**	**66.3**
	Dmean	**48.6**	**41.7**	**37**	**43.4**	**50.9**	**62.2**	**47.7**	**42.9**	**37.3**	**43**	**52.4**	**63**
8	*Dmax*	**56.6**	51.8	40.9	42.7	59.8	**66.9**	**55**	45.8	34.7	37	57	**68.5**
	Dmean	**41.8**	43.9	32.5	37.8	53.9	**60**	**44**	42.7	32.3	30	48.6	**61.6**
9	*Dmax*	**59.5**	54.1	36.2	37.4	51.7	**68.8**	**65.1**	58.8	42.2	46	59.5	**67.4**
	Dmean	**47.6**	46	30.6	30.7	41	**63.8**	**46.8**	50.5	38.1	42.8	51.5	**63.1**
10	*Dmax*	63.9	57.9	49.3	49.8	64.4	67.9	65.3	57.9	43.3	50.4	60.3	66.1
	Dmean	53	51.1	42.9	46.5	57.6	64.9	58.8	52.9	38.2	44.9	54.8	62.7
11	*Dmax*	**64.3**	42.7	42.4	42.5	**44.1**	**62.3**	**56.6**	**33.4**	34.6	34.5	32.9	**53.7**
	Dmean	**52**	36.1	35.3	36.4	**38.4**	**52.3**	**42.5**	**27.1**	26.9	24.7	24.6	**36.4**
12	*Dmax*	**63.8**	53.2	43.9	50.9	63.5	**63.8**	**63.8**	59	44.9	47.1	65.1	**67.9**
	Dmean	**49.3**	49.9	39	47.8	58.1	**60.3**	**60.3**	56.1	41.4	39.8	59.2	**63.2**
13	*Dmax*	**59.9**	40.4	35.5	43.8	65.2	**66.6**	**44.4**	**33.3**	24.7	32	**58.1**	**65.9**
	Dmean	**51**	35.9	31.3	37.7	54.8	**62.1**	**27.4**	**21.9**	17.2	19.1	**33**	**45.1**

Figure 6.4: Dmax and Dmean doses in Gys per patient for individual dental sites where microvascular imaging was completed. Doses in bold denotes edentate sites

	Mean outline area (μm²)	Mean total master segment (μm)
2011-12	36126.16 ± 18238.933	584.2677 ± 725.87350
2013-14	33271.90 ± 16669.091	624.9659 ± 688.79077
2015-16	38557.48 ± 14198.095	606.7047 ± 570.81960
Control	51478.29 ± 12803.900	1092.9195 ± 503.09707

Figure 6.5: Mean outline area and the mean total segment areas based on year of RT as well as the control group

	Control	Lateral incisor	Second premolar	Second molar	Overall p value Control vs RT group
Mean total branches length (μm)	3762.88 ± 1121.308	1867.54 ± 1039.239	1699.83 ± 909.365	1233.51 ± 857.105	**0.000**
Mean number of junctions	93.1000 ± 28.90110	74.6760 ± 43.76293	68.4000 ± 39.40007	47.8800 ± 31.64201	**0.0000**
Mean number of master segments	30.3800 ± 10.93793	23.2064 ± 21.38056	21.9600 ± 20.16745	12.5200 ± 13.96180	**0.001**
Mean outline area (μm²)	51478.29 ± 12803.9	39809.77 ± 17472.6	36237.54 ±16021.4	29123.85 ± 16027.7	**0.000**

Figure 6.6: Mean values for the varying dental sites for each MVD parameter identified to have high significance based on Mann Whitney U test.

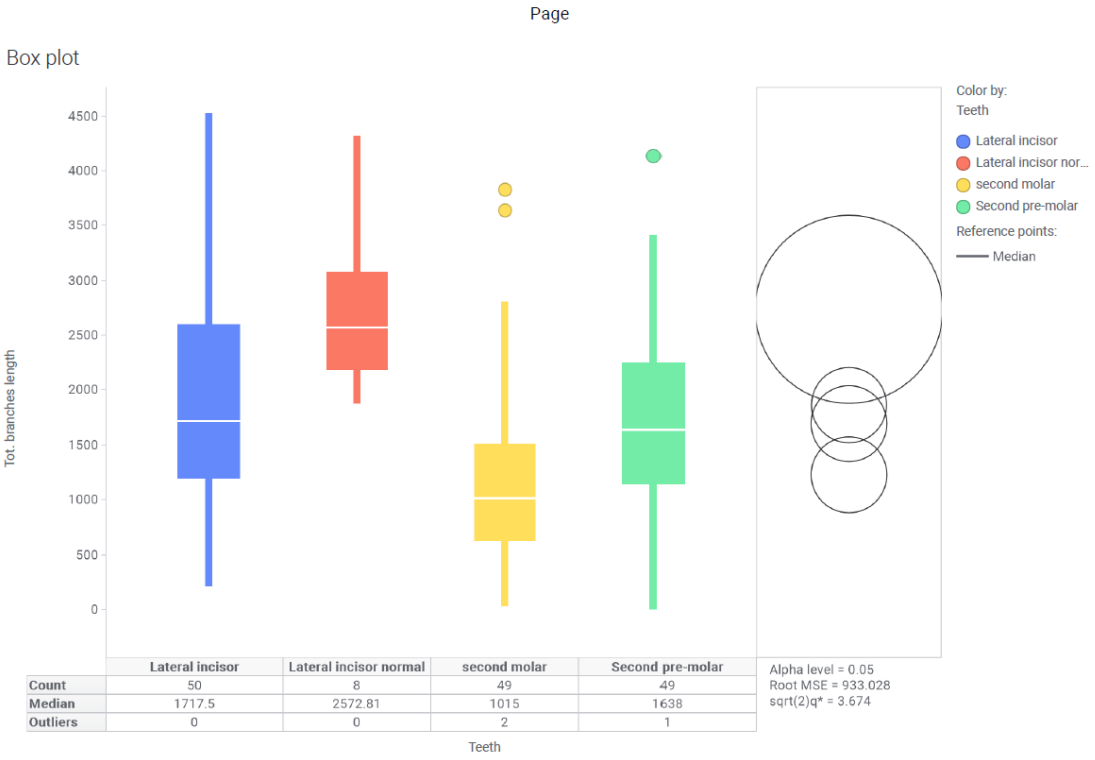


Figure 6.7: Box plot and comparison circles from TIBCO® Spotfire according to the total branch length. The median value indicated in the related box plot (white line) identifies a declining total branch length with more posterior dentition compared to the control group

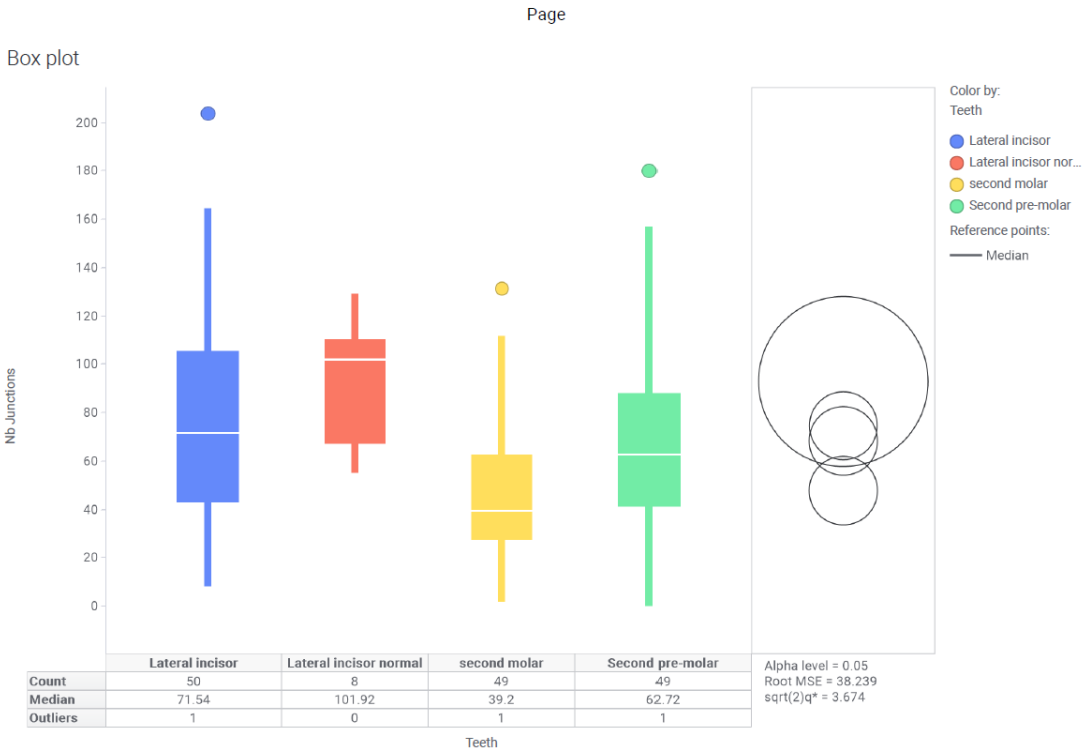


Figure 6.8: Box plot and comparison circles from TIBCO® Spotfire for the number of junctions. The median value indicated in the related box plot (white line) identifies a declining number of junctions with more posterior dentition compared to the control group

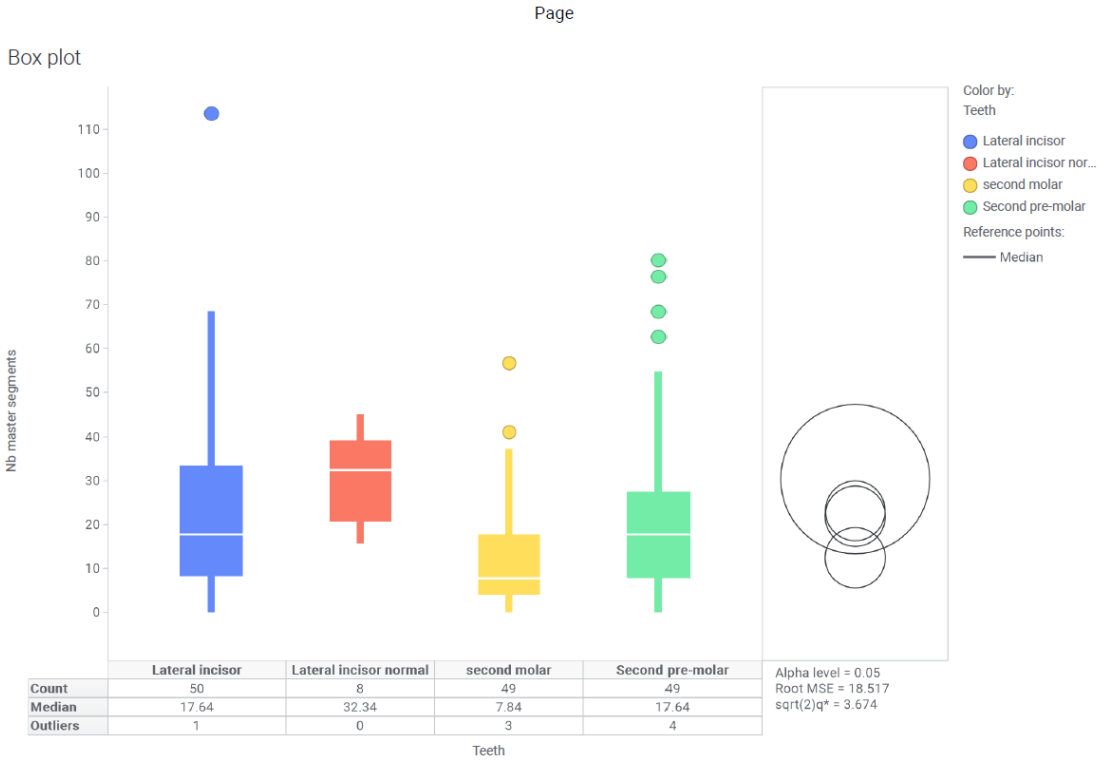


Figure 6.9**:** Box plot and comparison circles from TIBCO® Spotfire for the number of master segments. The median value indicated in the related box plot (white line) identifies a declining number of master segments with more posterior dentition compared to the control group

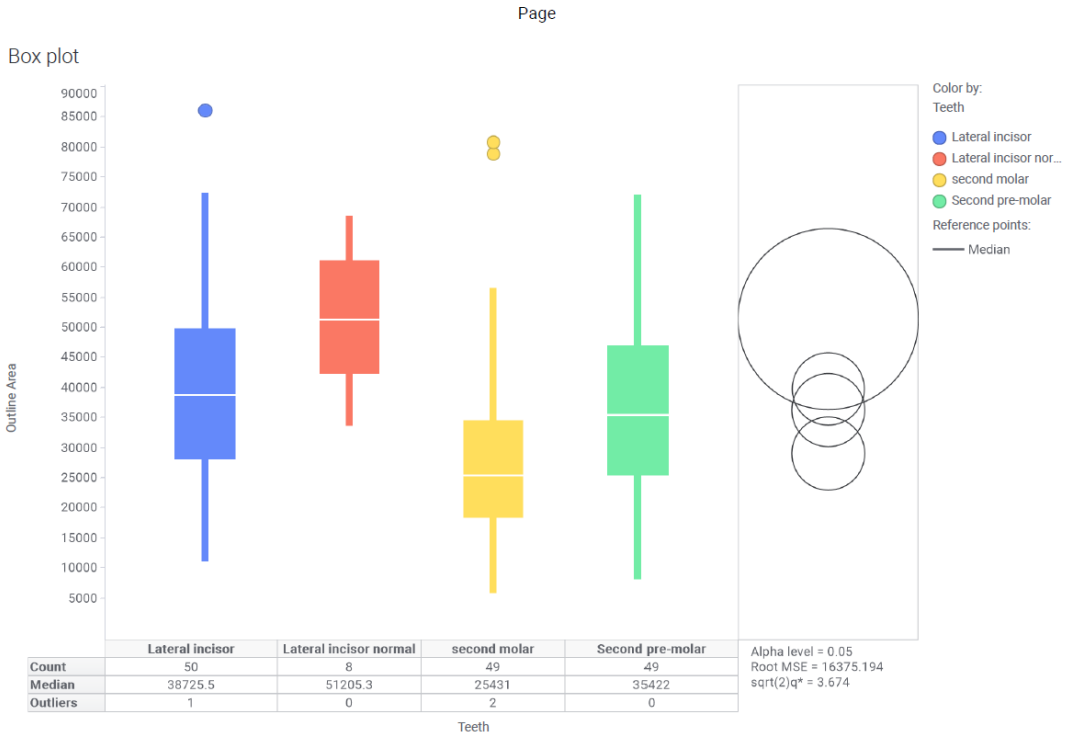


Figure 6.10**:** Box plot and comparison circles from TIBCO® Spotfire for the outline area. The median value indicated in the related box plot (white line) identifies a declining number of outline area with more posterior dentition compared to the control group

6.5 Discussion

Novel IMRT though targeted, exposes a larger amount of non-targeted tissues to irradiation (Rosenthal et al., 2008). No technology can entirely protect normal tissues from irradiation and patients will always experience some degree of radiation-associated toxicity (Strojan et al., 2017). Hence, in HNC it is unsurprising that ~50% of patients have late oral radiation toxicity (Denis et al., 2003). By conventional definition these effects are seen 3 months or more after the completion of RT (Strojan et al., 2017) and often progress over time (Barnett et al., 2009; Jellema et al., 2007; Langendijk et al., 2008). The development of irradiated tissue changes long term is often referred to as radiation induced fibrosis (RIF) and subsequent tissue death as radiation induced atrophy (S Delanian & Lefaix, 2004). For the latter, ORN is a common example of this. The concept of RIF is well recognised but understanding of its development has only become more apparent in recent years through advances in cellular and molecular biology (Denham & Hauer-Jensen, 2002; Hill et al., 2001; Stone et al., 2002).

In established fibrosis, excessive tissue induration and thickening is evident with limited or loss of function (S Delanian & Lefaix, 2004). Severity of RIF is multi-factorial (V Patel & M McGurk, 2017) with RT-related factors playing a key role. These factors include the total dose, the dose per fraction or fraction size, the RT volume and the schedule of treatment (Taylor et al., 1992). In addition, chemotherapy especially when concomitant with RT, might intensify certain acute and delayed reactions such as RIF development (Markiewicz et al., 1996). In the context of modern treatment scenarios, in many patients the damage caused by radiation is further aggravated by concurrent systemic cytotoxic agents (Pignon et al., 2009).

In mucosal tissue, fibrosis typically develops in the submucosa within weeks to months after beginning of the therapy (Fajardo et al., 2001; Small & Woloschack, 2006). Changes in the soft tissues post RT leading to late effects can be assessed in multiple formats (clinical, imagery, histopathology) (S Delanian & Lefaix, 2004). Clinically, four stages are described; pre-fibrosis, established fibrosis, late fibrosis, and atrophy (necrosis) (S Delanian & Lefaix, 2004). In contrast, histopathological changes commence immediately though clinically this may not be evident. The injured endothelial cells produce chemotactic cytokines that trigger an acute inflammatory response leading eventually to the release of tumour necrosis factor α, platelet-derived growth factor, fibroblast growth factor β, interleukins 1, 4, and 6, transforming growth factor $\beta 1$ (TGF-$\beta 1$), and connective

tissue growth factor (Dambrain, 1993; S Delanian & Lefaix, 2004). The subsequent effect leads to fibroblast and myofibroblast activation (S Delanian & Lefaix, 2004) which in turn leads to necrosis of microvessels, ischaemia, and atrophy of tissue (Dambrain, 1993; S Delanian & Lefaix, 2004). As for imagery, various methods have been employed to quantify microstructural, and metabolic data via positron emission tomography (PET), single photon emission tomography (SPECT), magnetic resonance (MR) imaging and MR spectroscopy. However, these investigations lack the ability to provide small volume microvessel differences and discrepancies which are vital to considering post-RT hard and soft tissue changes for the various anatomical structures of the oral cavity. Dose dependent mandibular bone vascular alterations related to chemo-RT has been demonstrated with the use of dynamic contrast enhanced magnet resonance image, known as DCE-MRI (JHNT-MRI-DC, 2016), but up until now, it has not been reported in relation to the soft tissues of the oral cavity. Hence, the use of an intra-oral microvascular endoscope is an ideal imaging methodology which can provide immediate non-invasive microvessel information. In the past, optical coherence tomography for *in-vivo* microvascular imaging of the oral cavity (buccal mucosa) during the stage of RT treatment has been used and been able to predict mucositis (Maslennikova et al., 2017) but no study has determined the microvasculature post-RT over time. The current study is the first to report dose dependent effect of RT in the microvascular anatomy of the oral cavity *in-vivo*. The use of teeth as reference points enabled us to correlate microvascular changes with site specific RT dose and therefore provided an element of accuracy and consistency.

The current study was able to highlight that increasing RT dose led to a negative impact on the microvasculature. As evident from the healthy volunteers' microvasculature atlas (A. P. Bastos, 2020; P. Bastos & Cook, 2016) no significant difference exists anterior or posterior. However, a clear significant difference was identified in the status of the microvasculature as it migrated posteriorly in the RT group (Figure 6.6-6.10). The one substantial factor changing was the RT dose which increased with the more posterior teeth (Figure 6.5). Hence an inverse relation was determined with increasing RT dose leading to depleting microvasculature. This study was able to quantify late changes in the gingival margin of patients who were previously treated with RT and for the first time establish *in vivo* RT dose-effect relationship. Furthermore, this study demonstrated that in both upper and lower jaws there was a significant decrease in all parameters (meaning decrease in vascularity) when comparing the microvasculature of the gingival margin of the posterior region (higher RT dose) with the anterior region (lower RT dose) and both these regions with the normal control group. Though vascular depletion was evident

minimal further information about the microvasculature could be determined as gingival capillary loops are observed from an axial perspective, hence its shape resemble "commas" and "dots" (Alessandro et al., 2009) which limits further morphological assessment.

Though the study was unable to determine an exact RT threshold dose for microvasculature changes worryingly this was evident even at low doses and raises the debate of whether the traditional 40Gy benchmark of irreversible RT changes is still applicable. The average Dmean for the anterior maxilla was 36.2Gy. Even at this dose significant changes in the microvasculature anatomy were noted. This lower threshold for the microvasculature depletion should not be a surprise considering RT induced mucositis typically begins at doses around 15-20 Gy (Scully et al., 2004) affecting 80% of patients (Trotti et al., 2003). At 30 Gy ulcerative mucositis occurs and subsequently evolves from asymptomatic focal hyperemia and edema to symptomatic patchy, then confluent desquamation (Scully et al., 2004). Hence, our findings are in line with the clinical findings commonly reported.

Capillaries are the most radiosensitive components of the vasculature and their histology manifests more radiation-induced morphological changes compared to the other types of vessels (Baker & Krochak, 1989; Fajardo et al., 2001; Rodemann & Blaese, 2007). Hence, the widespread RT to the oral cavity as evident from Figure 6.5 may partially explain the high incidence of RT late effects of mucositis, dysphagia and ORN this sub-site experiences and sustains (Turner et al., 2013). However, damage to the microvasculature of the oral tissue manifests as morphological or blood flow-related changes in the vessels, such as changes in vessel size and diameter, vessel architecture, and average blood velocity (Baker & Krochak, 1989; Fajardo et al., 2001). Eventually they begin to deplete due platelet-fibrin thrombi leading to obstruction and subsequent apoptosis, detachment and swelling of endothelial cells (Baker & Krochak, 1989; Fajardo et al., 2001). The concern of this process over the long term is significant fibrosis and atrophy based on the RIF model. This remains a real concern in the HPV positive OPC cancer with their favourable survival with the genuine risk of worsening late effects. However, in the current study we identified the possibility of a rebound response over time regarding the microvasculature. Though a general trend of decrease in vascularity was evident several years after treatment the sample size used in this study do not allow us to establish the exact effect of time in the microvasculature. Whether this rebound phenomenon is genuine cannot be fully determined from this study but is a legitimate possibility. Angiogenesis has been

noted in pathological situations such as chronic inflammation and tumour growth (Murphy et al., 2010; Scardina et al., 2011; Wang et al., 2014) and therefore could be a response to the RT over time.

6.6 Conclusion

This study demonstrated the feasibility of *in-vivo* microvascular imaging with RTOVI combined with CAA for the assessment of the late effects in the microvascular anatomy of patients subjected to RT.

From a clinical translational perspective, the study identified, and quantified morphological changes related to RT dose. These observations on microvascular imaging of dilated capillaries are in line with telangiectasia previously described (Reinhold et al., 1990). The importance of these findings highlights the status of the oral mucosa post-RT and provides early insight towards the fragile equilibrium of the microvasculature. Though evident this simply means little however, the progressive reduction highlights the potential role in how these changes may contribute towards the development of RIF and at its extreme ORN. Furthermore, the findings identified highlights that though IMRT is targeted the multi-beam approach leads to a wider exposure of tissues of the oral cavity. Though these RT doses may be low they are significant enough to lead to microvasculature changes. The impact of this in the long term are yet to be determined particularly in the current OPC cohort with their favourable survivorship.

6.7 Limitations

A number of limitations have been acknowledged in the current study.

6.7.1 Clinical

Firstly, the study population is small. Hence, any outlier patient is likely to influence the overall results. Furthermore, it is well recognised that microvasculature is impacted both positively and negatively via numerous factors such as age, medical conditions, medications and localised disease. In the latter, specific to the oral cavity this can range from periodontitis to mucositis. Additionally, the role of chemotherapy on the microvasculature is unknown. Some forms of chemotherapy are known to cause vascular changes, including endothelial dysfunction however, whether these changes are

exacerbated when combined with RT is unknown as all patients in this study received combined chemo-RT.

Though efforts were made to standardise the patient cohort certain factors cannot always be accounted for. The attempt to standardise is a significant reason for why the patient cohort is so small.

6.7.2 RTOVI

Capillaries in the attached mucosa or gingival mucosa are observed in an axial perspective and therefore offer a limited morphological assessment. In contrast, in other regions of the oral cavity such as the buccal mucosa the microvasculature run parallel to the surface providing a more detailed morphological microvasculature network image.

According to all CAA parameters there was a significant difference between the microvascular anatomy in the buccal and the lingual aspect of the gingival margin. The reason for this was not fully determined. Potentially, it may be related with the technical challenge of obtaining microvascular images from the lingual and palatal aspect (e.g. causing pressure artefacts) having an impact on image quality and subsequent outcome measurements.

Regardless of the limitations identified in this study, the results still provided valuable information for a small pilot study. To counteract these limitations, it would be advisable to consider a larger prospective cohort study to further validate these findings.

Chapter 7

The impact of IMRT on dento-alveolar microvasculature in pharyngeal cancer patients

7.1 Introduction

The synergy between irradiated teeth, soft and hard tissue remains the focal point in the development of ORN. Previous chapters have investigated and proposed the role of RT doses on the dentition and the oral mucosa identifying significant reasoning for their part in developing ORN. The final phase is determining how RT doses impacts the dento-alveolar bone. Rosenthal et al identified that IMRT led to significant higher RT doses to non-target areas compared to prior RT delivery systems (Rosenthal et al., 2008). This concern was reflected clinically with reports of rising rates of ORN following IMRT (Chang et al., 2007; Gomez et al., 2011). Detailed assessment of RT doses to the jaw identified in chapter 5 and in previous studies (Bak et al., 2016; Hansen et al., 2012; Parahyba et al., 2016) consistently shows large sections receiving in excess of 40Gy which has remained historically the threshold for developing ORN (Cooper, 2003).

The determination of dose related bone changes remains vitally important in the clinical management of HNC patients. Dental rehabilitation via dental implants is often feared as it is thought to increase the risk of ORN. However, in such challenging cases this remains the only option. With advances in RT delivery and accuracies in retrieving doses to specific sites this provides the opportunity to determine the threshold dose able to preserve sufficient bone regenerative ability to place dental implants without provoking ORN.

Simultaneously, the bone specimens can be examined to determine the pathophysiology of post-RT changes considering there remains two debated theories of ORN. Marx proposed the hypo-vascular, hypo-cellular and hypoxia concept where radiation would induce death of bone cells (endothelial, bone precursors, haematopoietic stem cells) with a compensation by less sensitive fibroblasts (R. E. Marx, 1983). Fibroblasts would then proliferate giving rise to a hypocellular or fibrotic appearance of irradiated bone. Marx therefore proposed adjuvant treatment using hyperbaric oxygen to compensate for these negative effects (R. E. Marx, 1983). In contrast, Delanian and colleagues suggested the concept of RIF (S Delanian & Lefaix, 2004) where a fibroblastic expansion at the expense of other cellular population and of vascular plasticity (vascular stiffening and lumen obliteration) would lead to tissue atrophy. Therefore, the authors proposed pharmacologic treatment (PENTOCLO) to improve blood flow, remove free radicals and encourage the loss of devitalised bone via sequestration and simultaneously laying of new bone. Both proposed pathophysiology mechanisms share a similar end point of vascular depletion causing avascular tissue necrosis. Of note, bone health and its vascularisation

are well known parameters (Liu et al., 2013) affecting the success and long-term predictability of dental implants.

Microvasculature assessment of irradiated jaws using either histology or other techniques have been previously performed in animal models (Bléry et al., 2015; Bodard et al., 2013; S. S. Deshpande et al., 2014; Sagar S Deshpande et al., 2014; Fenner et al., 2010; L. J. Poort et al., 2017; Sønstevold et al., 2015; Xu et al., 2012; Yachouh et al., 2010). These studies have demonstrated that in general radiation therapy induced a reduction in bone vascularization but fell short in indicating radiation threshold dose of significant changes to the bone's microvasculature. Indeed, commonly, total RT doses are reported rather than site specific doses which does not allow understanding the effects of different doses within the same individual. Furthermore, vascularization is evaluated in large bony specimens (e.g. whole mandibular ramus) which do not necessarily reflect the area of ORN vulnerability which is the dento-alveolar segment. This is of particular importance considering this is where the dentition and dental implants are placed and where ORN most commonly occurs. Furthermore, assessing the inferior alveolar artery and the periosteum does not directly reflect the vascularity of the cancellous bone. Finally, in several studies bone vascularization was assessed only in large vessels and not within bone's microvasculature. In order to quantify radiation-induced damage to the vascularization of bone, it is vital to quantitatively assess the effect of irradiation on trabecular bone's microvascular network.

This study aimed to assess the relationship between varying IMRT doses in pharyngeal cancer patients and the subsequent vascular changes in the dento-alveolar region of human jaws including microvasculature density (MVD), vascular enlargement and obliteration.

7.2 Methods

7.2.1 Ethics

Research approval for this study was provided by the Research Ethical Committee, London, England (16/LO/1797).

7.2.2 Case selection

A total of 18 patients were included in this study with a 1:1 ratio of controls (n=9) versus post-RT patients (n=9). Patient demographic comparison between the two groups were

comparable in gender, mean age (control 58.6 years, post-RT 62.2 years) and smoking status with near identical sub-groups.

The control cohort was selected retrospectively from patients who had previous dento-alveolar resection for either previous oral cancer with a minimum clearance margin of 1cm from the tumour advancing front or benign neoplasms such as ameloblastoma. Control cases were identified from the official HNC multi-disciplinary patient registry and age-matched within the expected HNC age-range in order to mitigate against age-related variation of bony architecture. Specimens selected required clear evidence of tumour free margins as approved by a consultant histopathologist.

The prospective patients for the study group were selected from the HNC dental rehabilitation clinic. Patients requiring dental implant rehabilitation, having previously (mean: 5.5 years) had IMRT treatment at GSTFT were included to allow for accurate RT dose retrieval. All patients had biopsy proven squamous cell carcinoma either of the nasopharynx, oropharynx or hypopharynx treated with curative intent. Exclusion criteria included non-IMRT patients or previous history of ORN or bisphosphonate use.

7.2.3 Dental implant surgery and bone biopsy retrieval

Clinical and surgical treatment were undertaken by 2 specialist oral surgeons. Surgery was provided as clinically required (local anaesthesia, local anaesthesia and intravenous sedation, general anaesthesia) and dental implants placed as clinically planned in both the maxilla and mandible.

Bone core biopsies were taken at the time and as part of the implant surgery. The dental implant procedure included a full mucoperiosteal flap. The implant osteotomies were conducted under copious irrigation with sterile saline. Initially, implant preparation was made with a 3.0 mm bone trephine bur (2.5 mm inner diameter) (Meisinger Trephine Kit, Meisinger Germany, Manufacturer Part No: 7120). Post-trephine a bone core pick-up instrument allowed simple and atraumatic specimen retrieval. The aim was to obtain a bone core length of two-thirds of the desired implant length to be placed. This was to allow appropriate osteotomy of the apical third to achieve primary stability of the implant upon placement. Placement of implants were limited by anatomical constraints such as the inferior alveolar nerve canal or the maxillary sinus or simply lack of bone height. A minimum of 6mm bone core length was achieved in all cases. Each bone core was placed into individual vials filled with 10% buffered formalin and labelled to their equivalent

dental site. Subsequently, implant osteotomy was completed in line with the Astra Tech Implant system protocol. Implants placed were either 4.0mm or 5.0mm in width or 9.0mm or 11.0mm in length. All dental implants underwent a one-staged surgical procedure with placement of a height appropriate healing abutment.

7.2.4 Dental dosimetry

Dental RT doses were retrieved from the Monaco® treatment planning system. The exact same methodology and parameters described in 5.2.4 (Dosimetry) were applied to retrieve RT doses (Dmax and Dmean) (Figure 7.1). Dose-volume histogram (DVH) data was exported per tooth contour per patient and the mean dose (Dmean) and maximum dose (Dmax) calculated using the DVH metrics package (R software).

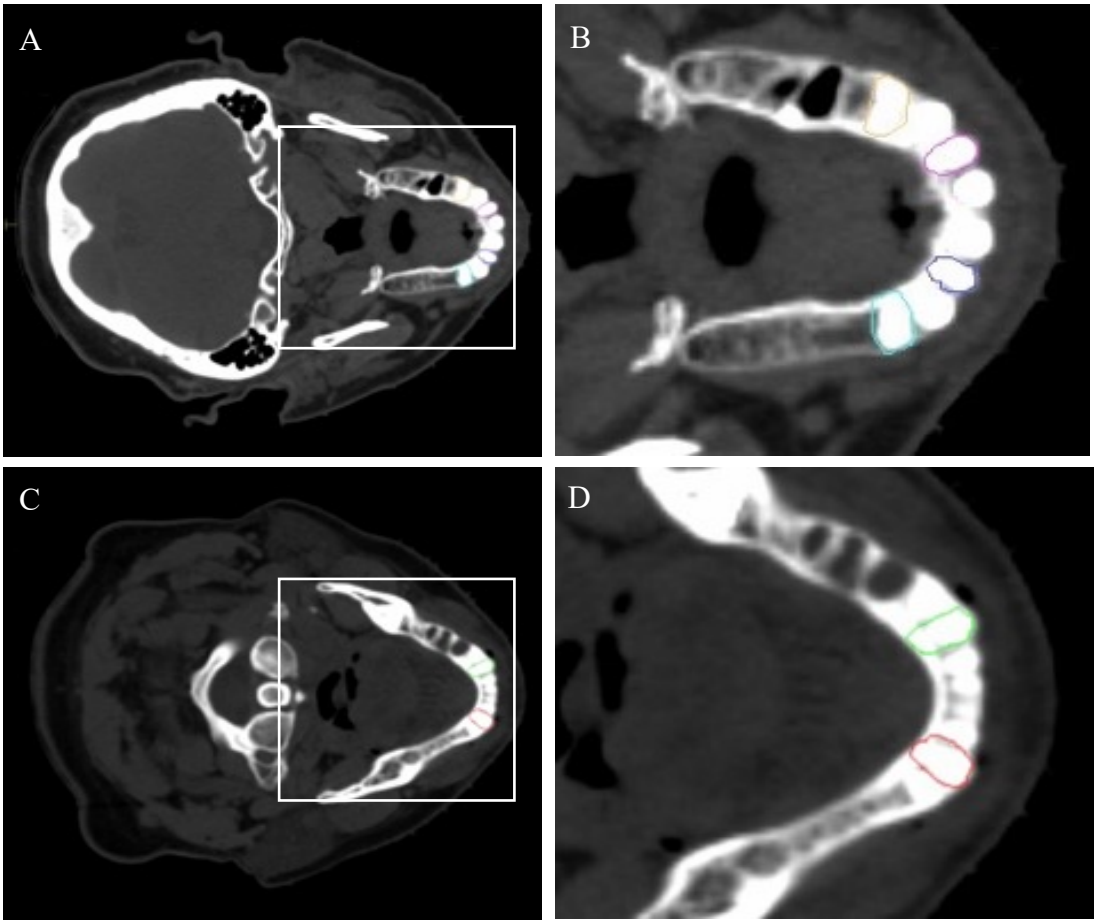


Figure 7.1: CT scan axial view for the maxilla (A) and mandible (C). Images B and D are zoomed images of the jaws showing the contouring of the teeth for patient 11.

7.2.5 Processing of the bone biopsies and control specimens

Following removal, bone cores were immediately fixed in 10% (v/v) buffered formal saline for 24 hours, then decalcified whole in 10% (v/v) buffered formic acid. Endpoint testing was undertaken by visualisation using a Hewlett Packard Faxitron (model 43855A). Decalcified tissue was processed and embedded in paraffin wax. Sections of 5 μm were cut and mounted on positively charged slides. The sections were deparaffinised in xylene, dehydrated in 99% (v/v) industrial methylated spirit, and rinsed in running tap water. All sections were routinely stained with hematoxylin and eosin. Tumour-free bone resection margins from non-irradiated mandibulectomy and maxillectomy specimens was used as control material. Control tissue blocks were selected following retrospective case review by a consultant histopathologist. All control material was fixed, decalcified, endpoint tested, processed, sectioned and stained the same way as bone core specimens. CD31 (Dako M0823, Clone JC70A. using Roche antibody diluent 251-018 at 1:25) immunohistochemistry was undertaken was on an automated platform (Benchmark Ultra; Ventana Medical Systems, Tucson, AZ, USA). Antigen retrieval, antibody incubation and chromogen application were performed according to manufacturer instructions.

7.2.6 Microvasculature measurement of the bone biopsies and control specimens

Stained slides were acquired with a NanoZoomer Digital slides scanner (Hamamatsu) (Figure 7.2). Whole slides digital images were viewed with NDP View (Hamamatsu) to extract representative images for analysis (3 images/slides, RGB, 1496x1496 px = 680 X 680 μm). Images were saved as tagged image format (tif) file, imported in ImageJ (NIH) and analysed in batches using a specifically developed ImageJ macro. Since the intensity and quality of staining varied widely within our sample set, simple threshold-based features extraction algorithms (Veschini et al., 2011) were insufficient to unbiasedly evaluate all the images. To overcome this limitation, we developed a novel ImageJ macro relying on a machine learning core which takes advantage of the WEKA segmentator plugin (ImageJ) (Arganda-Carreras et al., 2017). In brief, firstly .tif images were pre-filtered (adjust brightness contrast, unsharp mask). Pre-filtered images were used to generate a training set for the WEKA segmentator plugin composed of a single image stack containing 90 slices (250X250 px). The trainer stack was loaded into the WEKA segmentator and images were manually annotated to train it. We generated two models, the first to distinguish vessel (CD31 positive structures) and the second to distinguish soft tissue juxtaposed to bone lamellae from tissue or background. We applied the WEKA segmentator to generate segmentation maps for all sample set. Segmentation maps were

thresholded and particle analysis performed to generate a result table. The macro returned a comma-separated values (.csv) file containing number of vessels per image, calibre of each vessel and other geometric features useful to filter out non-meaningful results (e.g. very large structures representative of dilated vascular sinuses rather than micro vessels). Data were pre-selected in Microsoft Excel to filter out all non-microvascular structures and to establish radiation doses categories across patients. Results were exported in GraphPad Prism for statistical analysis and plotting.

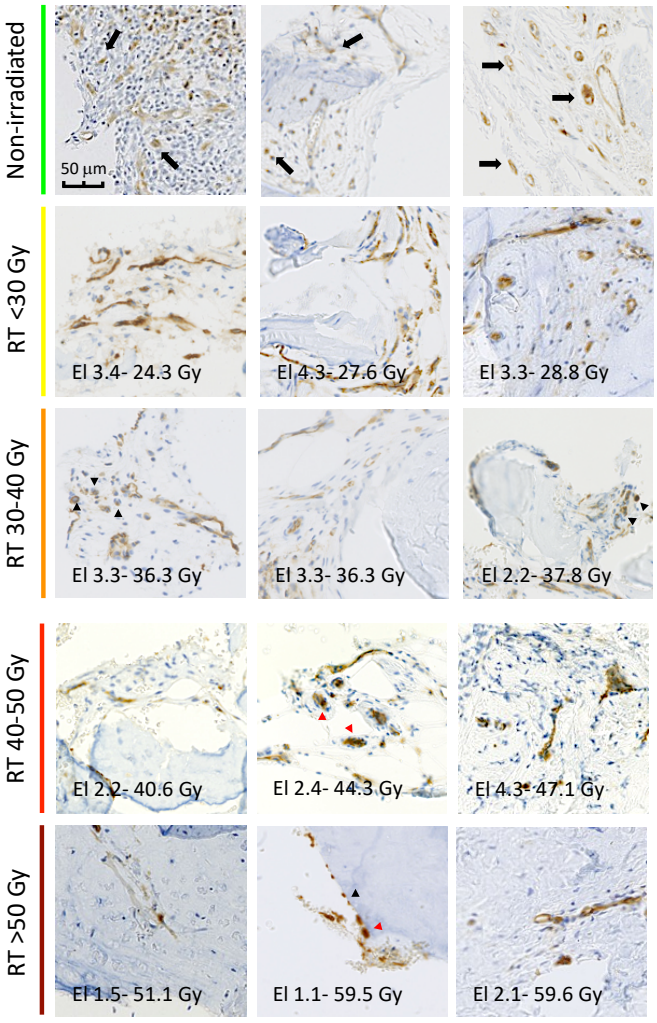


Figure 7.2: Representative photomicrographs of different samples which received the indicated radiation. Dose (text inset) and laid out according to the categories used for quantification (rows). Arrow indicate patent vessels; black arrowheads indicate obliterated micro-vessels and red arrowheads indicate obliterated medium/small vessels.

7.3 Statistical analysis

Statistical analysis was performed with GraphPad Prism. Student's t-test was utilised to compare two datasets for which $p<0.5$ was considered significant, $p<0.1$ and $p<0.001$ were considered highly significant.

In addition, one-way ANOVA was used when comparing multiple datasets followed by Bonferroni post-hoc test for multiple comparisons (Benjamini et al., 2006). $p<0.5$ was considered significant, $p<0.1$ and $p<0.001$ were considered highly significant. Pearson's correlation analysis was performed to evaluate co-variance of MVD with radiation dose. We reported r and R^2 values and considered $p<0.0001$ as highly significant.

7.4 Results

A total of 18 patients were included in this study with a 1:1 ratio of controls (n=9) versus post-RT patients (n=9). Patient demographic comparison between the two groups (Figure 7.3) were comparable in gender, mean age (control 58.6 years, prospective 62.2 years) and smoking status with near identical sub-groups.

Within the prospective group, the oropharynx (n=5/9, 56%) was the most common tumour sub-site. All but two patients had a total RT dose of 65Gy and adjuvant chemotherapy.

A total of 23 bone cores were retrieved from the prospective group (Figure 7.4) with a range of dental sites extending no further than the 2nd premolar region in both jaws. Surgical site dosimetry is presented in figure 7.4 with the average, median, range and standard deviation for Dmax, Dmean and Dmin presented in figure 7.5.

Qualitative histological evaluation

Qualitative observation of histological samples (Figure 7.3) showed dramatic changes that were induced in the dento-alveolar bone upon RT. Control samples demonstrated evenly distributed micro-vessels of different calibre (Figure 7.3A, arrows), these micro-vessels are curvilinear and patent without signs of engulfment or collapse. In samples which received mild irradiation doses (Figure 7.3) micro-vessel numbers were noticeably reduced, with a clear reduction in the miniscule terminal capillaries and high incidence of obliterated lumens (Figure 7.3 arrowheads). Finally, samples which received high irradiation doses were clearly hypo-cellular; remaining cells were elongated and

embedded in a fibrous matrix. In these samples, almost all micro-vessels were lost and remaining large ones were often engulfed or collapsed (red arrowheads).

Quantitative evaluation of MVD and vessel morphology

The MVD was measured for each sample using the image analysis pipeline (Figure 7.6B). On analysing the data in bulk (non-irradiated vs irradiated), our findings corroborated with previously reported results, indeed, we found that overall, the MVD of irradiated patients was markedly reduced (Figure 7.6A, 653.5 ± 326.3 vs 387.7 ± 209.4, p= 0.0034).

In order to correlate MVD with radiation doses, the data was plotted according to RT doses (Figure 7.7A) and a linear regression analysis performed. It was observed that MVD and RT doses were inversely correlated with an r=-0.5168 (R^2= 0.2671, p<0.0001). To determine whether it was possible to identify a "safe" RT dosage which could prevent side effects and allow placement of dental implants, the data was sorted into five categories (Figure 7.7A). This analysis demonstrated that in sites which received less than 30Gy RT approximately 77% of the vessels were preserved (Figure 7.7A, 0 Gy= 653.5 ± 323.6 vs <30 Gy= 504.6 ± 222.4, p<0.001). By contrast, exposure to higher doses 40-61% of the micro-vessels were lost (Fig 4A, 30-40 Gy = 392.2 ± 152.5, 40-50 Gy = 309.6 ± 189.2, >50 Gy = 253.8 ± 191.8). The distribution of small micro vessels (<20 mm) across three categories was plotted (Figure 7.6C 5-10, 10-15, 15-20 mm). It was observed that in all irradiated categories, the smallest (5-10 mm) micro vessels were markedly reduced with a more pronounced drop with high RT doses. Importantly, these tiny microvessels are those primarily responsible for the trophic functions, thus a reduction in these is strongly indicative of increased tissue irradiation

Overall, these results indicate that RT, independently from the dosage, induces vascular alterations and "pruning" of small calibre micro vessels, indicating that doses up to 30 Gy seem to preserve sufficient vascularisation (~77% in comparison to control) and tissue architecture (Figure 7.2). Overall, the 30-40Gy category display a clear drop in MVD (-40%) and pruning of small microvessels. Nonetheless, in some areas the vasculature along with tissue architecture seems preserved, thus representing a worst profile than the first two categories (0-30 Gy), but not as bad as the latter two (>40 Gy).

Group	Patient No	Gender	Age	TNM7	Site	Side	Treatment	Total RT dose (Gy)	Chemotherapy drug	Smoking	Medical Conditions
Control	1	M	48	T2N0	Retromolar	L	N/A	N/A	N/A	Y	Nil
	2	F	50	N/A	Mandible ameloblastoma	L	N/A	N/A	N/A	N	Nil
	3	F	72	T4N0	Alveolus	R	N/A	N/A	N/A	N	Nil
	4	M	55	T4N0	Maxilla	L	N/A	N/A	N/A	Y	Nil
	5	F	58	T4N2	Alveolus	Midline	N/A	N/A	N/A	N	Nil
	6	M	47	N/A	Mandible ameloblastoma	Midline	N/A	N/A	N/A	N	Nil
	7	M	72	T4N0	Mandible	R	N/A	N/A	N/A	Y	Nil
	8	M	66	T4N2	Mandible	Midline	N/A	N/A	N/A	Y	Nil
	9	M	59	T3N0	Maxilla	R	N/A	N/A	N/A	Y	Nil
Prospective	10	M	53	T1N1	NP	N/A	RT	54	N/A	N	Nil
	11	M	55	T2N2	OP	R	CRT	65	Cisplatin	Y	Nil
	12	M	46	T3N2	OP	R	CRT	65	Carboplatin	N	Nil
	13	F	66	T4N2	HP	L	CRT	65	Carboplatin	Y	Nil
	14	M	58	T4N1	HP	N/A	RT	60	N/A	Y	Hypercholesterolemia
	15	M	72	T3N2	OP	R	CRT	65	Cisplatin	X	Nil
	16	F	65	T4N1	OP	L	CRT	65	Carboplatin &5FU	N	Nil
	17	M	61	T1N2	OP	R	CRT	65	Cisplatin	Y	Emphysema Hypertension
	18	M	78	T3N2	OP	Midline	CRT	65	Carboplatin	N	Nil

Figure 7.3: Demographic spread of both the control and prospective group (OP - oropharynx, NP – nasopharynx, HP – hypopharynx)

Patient No	No of sample sites	Implant Site 1			Implant Site 2			Implant Site 3			Implant Site 4			Implant Site 5			Implant Site 6		
		Dmax/Dmean/Dmin			Dmax/ Dmean/Dmin			Dmax/ Dmean/Dmin			Dmax/ Dmean/Dmin			Dmax/ Dmean/Dmin			Dmax/ Dmean/Dmin		
10	3	UL2			UR5			LR5			-			-			-		
		46.8	40.6	35.1	56.2	51.1	44.9	49	39.9	34.6	-	-	-	-	-	-	-	-	-
11	6	UR2			UL2			UL4			UR4			LL3			LR3		
		48	42.9	38	40.3	37.8	34.6	44.3	39.5	31.6	54.7	50.5	41.7	43.7	36.3	30.4	51.3	47.1	41.3
12	1	LL4			-			-			-			-			-		
		32.4	24.3	17.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	6	UL4			UL2			UR2			UR4			LL3			LR3		
		33.1	25.6	18.5	37.1	31.5	24.2	35.4	29.7	22.3	31.9	25.9	21.1	40.7	36.3	30	31.2	27.6	23.9
14	1	LL3			-			-			-			-			-		
		32.9	30.3	25.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	2	LL3			LR3														
		50.7	44.7	37	61.5	50	45.1	-	-	-	-	-	-	-	-	-	-	-	-
16	2	UR1			UL1			-			-			-			-		
		61.8	59.6	58.3	61.3	59.5	56.5												
17	1	LL3			-			-			-			-			-		
		35.4	28.8	25.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	1	UR3			-			-			-			-			-		
		28.9	24.9	20.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

RT >50 Gy RT 40-50 Gy RT 30-40 Gy RT <30 Gy

Figure 7.4: Prospective cases with the dental site where bone core was taken and RT dose (Dmax, Dmean, Dmin)

	Average (Gy) (standard deviation)	Median (Gy)	Range (Gy)
Dmax	43.9 (10.6)	43.7	61.8-31.2
Dmean	38.4 (10.8)	37.8	59.6-24.3
Dmin	33.0 (11.4)	31.6	58.3-18.5

Figure 7.5: Average, median and range dental dosimetry values at Dmax, Dmean and Dmin

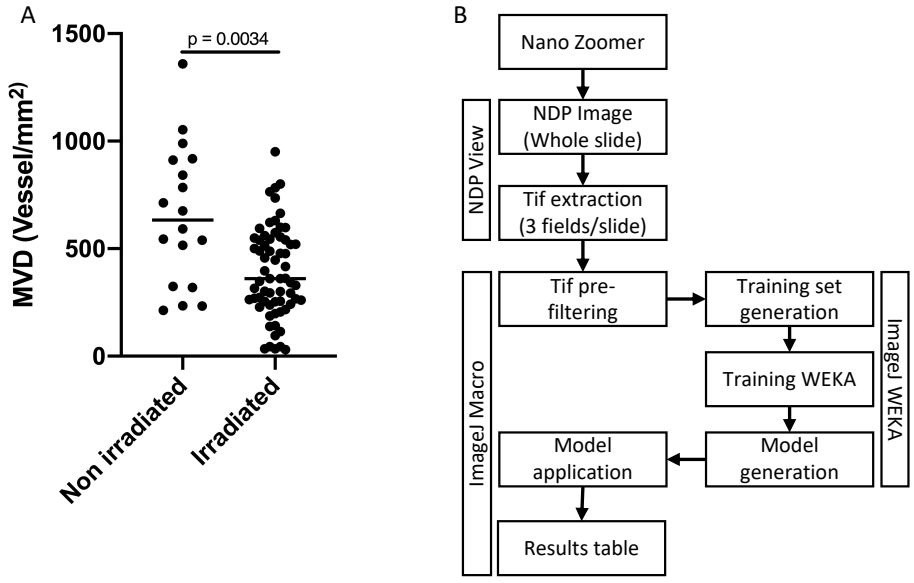


Figure 7.6: A) MVD of irradiated vs non-irradiated sites. Black line represents the mean of the group B) schematic representation of the image analysis pipeline indicating the software used for each step.

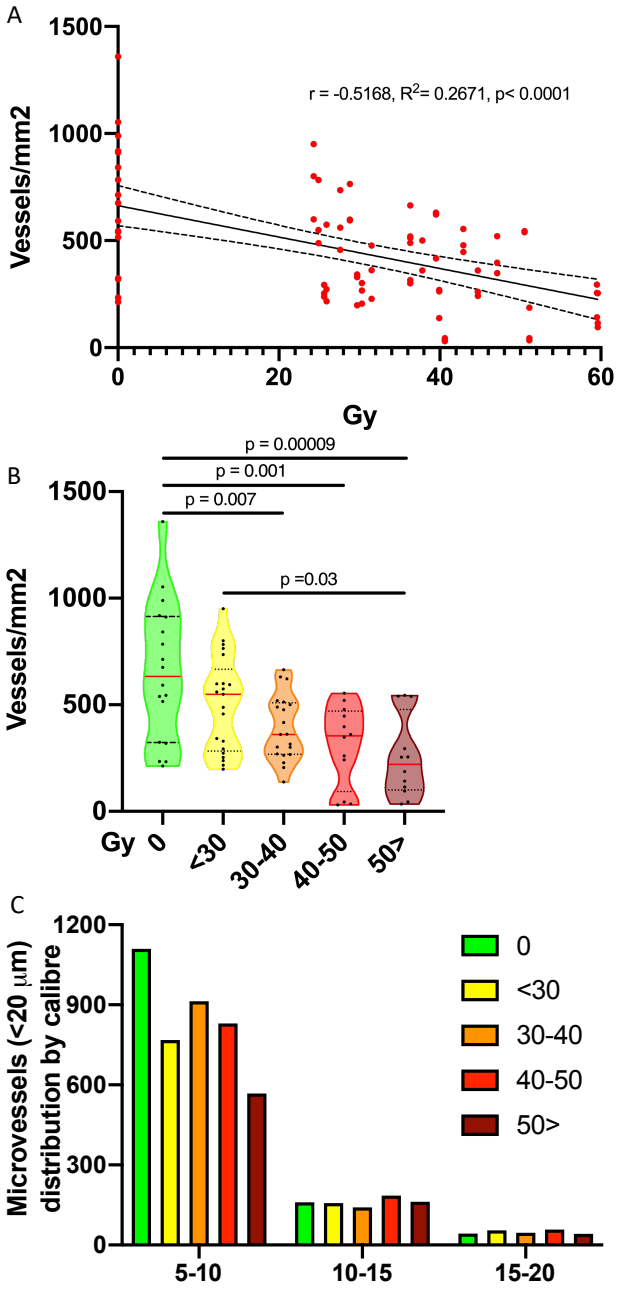


Figure 7.7: A) Linear regression analysis of MVD against RT dose. Black line is the best extrapolated fitting line and the dashed lines represent the 95% confidence bands. B) Violin plot of the five categories, red lines represent the median for the group (50th percentile), dashed black lines are the 25th and 75th percentiles. C) Distribution (number of values) of micro vessels across the five RT categories (colours) and 3 calibre categories smallest, medium and medium-large capillaries (5-10, 10-15, 15-20 microns respectively).

7.5 Discussion

The current study has confirmed the fundamental principles that RT leads to vascular depletion with a substantial finding that a significant threshold is 30Gy. Historically, the threshold was considered 40Gy which led to impairment of bone regeneration capacity (Schoen et al., 2007). Progression and failure of this process commencing at 40Gy risked the development of ORN (Cooper, 2003). Identification of this lower threshold of 30Gy is a clinical concern. Patients with tumours of the oropharynx, nasopharynx and hypopharynx all routinely receive doses in excess of this to the jaws (Bak et al., 2016). This new lower threshold should be no surprise when considering surrounding structures such as enamel (de Barros da Cunha et al., 2017), dental pulp (Garg et al., 2015) and submandibular glands (Murdoch-Kinch et al., 2008) all undergo irreversible damage in RT doses less than 40Gy.

The study also recognised inflamed microvasculature in the irradiated group, though all patients completed their RT over 12 months prior. This finding highlights the sustained and residual effects of RT and are aligned to those described in the early phase of the pro-fibrotic model proposed by Delanian & Lefaix for ORN (S Delanian & Lefaix, 2004). In the first phase, known as the pre-fibrotic phase, non-specific chronic local inflammation, destruction of endothelial cells and vascular thrombosis is seen (S Delanian & Lefaix, 2004). Subsequent progression leads to abnormal fibroblastic activity and therefore poorly vascularised and cellularised tissue sustained for decades after radiotherapy (S Delanian & Lefaix, 2004). The eventual quantitative reduction of microvasculature with simultaneously hypoperfusion provides poor healing capacity (R. Marx & Tursun, 2012).

The trend seen in the current study has been repeatedly reported in animal studies (S. S. Deshpande et al., 2014; Poort et al., 2017). Poort LJ et al showed in a mini-pig study the degree of vascular change was dose-dependent affecting even the diameter of the inferior alveolar artery (Poort et al., 2017). Vascular damage led to an increase in fibrotic tissue and high osteoclastic activity leading to an increase in fibrosis, resorption lacunae, necrosis, and the woven-lamellar bone ratios with higher radiation dosages (Poort et al., 2017). These findings were supported in a rat model, where a reduction in vessel volume fraction and vessel number were seen when compared to non-irradiated controls (S. S. Deshpande et al., 2014).

Studies on patient samples are limited and have been typically performed on tissue specimens derived from ORN lesions or oncological resections in previously irradiated patients (Bras et al., 1990; Curi et al., 2016; R. Marx & Tursun, 2012; McGregor & MacDonald, 1995). The current study is the first, to the authors knowledge, to report on human jawbone with the microvasculature

based on site specific RT doses rather than using total clinical dose. The use of specific dose provides a more accurate and true representation of the status of irradiated bone. In contrast, the use of ORN resections to assess vascularity is likely to identify profound lack of blood supply, hence, leading to bone death. The study by Dekker et al comparing irradiated mandibles versus controls using CD34 antibody stain to detect endothelial cells identified similar findings (Dekker et al., 2018) to the current study. They found mean vascular perimeter and mean vascular diameter were higher in samples with a local radiation dose of ≥50 Gy, whereas the percentage of small vessels was lower (Dekker et al., 2018). Based on these findings, Dekker et al concluded radiation dosages higher than 50 Gy mainly affect the smaller vessels (Dekker et al., 2018). In contrast, our study identified this threshold to be in excess of 30Gy. Though this study replicates a similar theme to ours regarding microvasculature a number of limitations were evident. Firstly, the patients had undergone differing RT delivery systems which is known to lead to differing RT doses to the jaws (Rosenthal et al., 2008). Furthermore, their study-estimated doses, in contrast to ours for which we were able to retrieve the exact RT the bone specimen has received. Also, their study cohort underwent HBOT, which is a proposed treatment to combat radiation vascular changes, which may have influenced the vascularity.

The wider perspective remains the impact of vascular depletion on the tissues over time on the background of cancer survivorship. This is of particular concern in the HPV positive OPC patient. Dental dosimetry shows these tumour groups with IMRT receive doses >30Gy to the jaws (Bak et al., 2016; Hansen et al., 2012; Parahyba et al., 2016; Rosenthal et al., 2008) (Chapter 5) . In addition, HPV positive OPC have significantly improved overall- and disease-free survival compared to site- and stage-matched HPV-negative tumours (Ang et al., 2010; Pytynia et al., 2014). In addition, one should consider the remarkable increase in the incidence of OPC in the past three decades associated with HPV infection (Chaturvedi et al., 2013) with the current trajectory estimated to lead to 50% of all HNC being OPC (Chaturvedi et al., 2011). This group of patients are likely to be vulnerable to ORN based on the current findings or have limited dental rehabilitation options if the jawbone vasculature status is one-way motion of depletion.

One important factor often overlooked is the role of chemotherapy and its impact on the tissues. It is estimated the impact of concurrent chemotherapy is equivalent to receiving an additional 10Gy of RT (Kasibhatla et al., 2007) and this could be a potential reason for the lower RT threshold of 30Gy identified in this study. Historically, chemotherapy was rarely used in HNC, however, it is prescribed routinely in pharyngeal cancer. The exact impact of chemotherapy in the current study cannot be fully determined due to numerous factors such as the variance in

drug, regime, dose and timing of prescription. However, it's use and impact on microvasculature is a feasible concept and may partly explain the lower RT threshold identified in the current study.

7.6 Conclusion

The current study identified that RT is impactful on the MVD. Increasing RT leads to depletion of MVD with the dose above >30Gy being the key threshold.

MVD in irradiated bone showed dilated and inflamed MVD highlighting long standing and chronic reaction to RT. These findings are in line with the current accepted pathophysiology of radiation induced fibrosis.

7.7 Limitations

Though this remains the only study to report human dento-alveolar bone MVD status post exact RT dosimetry a number of limitations are present. Firstly, the patient cohort and bone core yield are limited.

Secondly, there is a clinical bias based on patient selection. Implant placement within our department is often agreed for patients deemed to be at low risk of ORN. Hence, implants are limited to the premolar regions as the most posterior site and in areas not estimated to exceed the 40Gy site. Though a number of sites did exceed the 40Gy mark the overall average and median Dmean were <40Gy.

Thirdly timing has been shown to lead to depletion in MVD both in this chapter and the preceding one. The current study did not consider this factor due to limited number of patients and cores. A larger number of both would be ideal.

Finally, bone cores were retrieved from a range of regions in the mandible and maxilla. Bone architecture, mineralization, paucity and vascularization varies both between jaws and within the jaw from anterior to posterior. Hence a larger number of cores would be required to incorporate these variances.

Chapter 8

Conclusions and summation

8.1 Conclusion

The current thesis has explored the dental status as well as the IMRT doses to the teeth, mucosa and bone all of which are recognised to have a significant role in developing ORN.

Considering the starting point of OPC compared to their fellow HNC patients they immediately are seen to be a sub-group having a minimum functional dentition of >21 teeth. However, unlike many of the other sub-sites, OPC is well recognised to be have the sub-division of HPV positive and HPV negative tumours. The former has significantly more teeth than the latter. Once separated by this sub-division the HPV positive group is comfortably above the 21 teeth functional threshold with an average of 22.3 teeth while the HPV negative group have on average 19.0 teeth. Often the difference in teeth is related to the posterior dentition.

The presence of more teeth inadvertently means an increased risk of ORN. Though OPC patients have more teeth than other HNC patients it's the subsequent post-RT effect to them which is concerning. In the HPV positive group, a difference of 9 teeth was identified at the pre-RT phase between the age decades of 55-64Y to 75-84Y. Hence, this finding suggests regardless of any cancer treatment there is a natural tooth loss of 9 teeth over a 20-year period. This finding alone carries huge clinical significance. Considering that the 55-64Y group is the peak age incidence for OPC and that HPV positive patients have favourable outcomes it is completely conceivable that as this tooth loss trend continues or even expedites with the addition of RT. However, now there is the added threat of ORN and with tooth loss commonly affecting posterior teeth first this highlights both an elevated risk as well as the existence of a life-long threat. Compare this trend to HPV negative patients who in 2 decades will only lose 2 teeth on average with an overall 3-year survival of only 57.1% (Ang et al., 2010). Hence, ORN as a late effect has limited bearing on this group and if it does occur is often short lived as opposed to the HPV positive group.

The clinical conundrum for the dental oncologist is prescribing treatment at the pre-RT phase on the balance of survival, development of late effects and oral function. In the OPC group this has crudely been done via HPV positive status, however this does not accurately predict outcomes with some HPV positive patients having poor survival outcomes and equally some HPV negative patients having long survival periods. More recently, the use of TILs has been proposed as a better prognostic predictor and has been validated via several studies. Though TILs does not have a direct impact on the dental status of OPC patients the ability to predict long term outcome and incorporate this into clinical decision making at the dental assessment stage is a potentially valuable tool.

Having established that OPC patients have more teeth the subsequent RT doses to the region are a cause for concern when considering ORN. Doses to the posterior dentition on the ipsilateral tumour side receive near total amount. In the larger tumour group the same hold true for contralateral side. Currently this applies to a large population group within OPC considering that HPV positive disease is an indolent disease often presenting as large painless neck lump. Currently this is considered advance disease and RT is delivered to the neck bilaterally. Subsequently the jaws become incorporated within the field and with a large area of the dento-alveolar segment receiving a substantial dose (>40Gy). With enlarging tumour size and nodal involvement, the coverage of the jaw increases with more teeth receiving a dose above the 40Gy mark. In profiling patients via RT doses based on explanatory attributes males with a right-sided BOT tumour with staging of T4N2 receive the highest dose and would therefore be considered to have an elevated ORN risk.

Though a focus is placed on the 40Gy ORN threshold this benchmark may need re-evaluating. In both oral mucosa and dento-alveolar bone significant depletion of microvasculature were seen at the lower dose of 30Gy. Arguably the most important tissue for assessing an end point of ORN is the bone itself. This phenomenon could be due to the routine use of chemotherapy which has a synergistic impact on tumour tissue itself but equally could be negatively impacting the hard and soft tissue. Considering this lower dose of 30Gy, based on the contour study, irrespective of TNM classification most of the dentition received close to or in excess of this dose. If this threshold was to hold true, the clinical concern is apparent that essential the whole dentition across both jaws is 'at risk' of ORN on the background of long-term survivorship.

8.2 Summation

In the past two decades, two significant changes have had an impact on the HNC landscape. Firstly, there has been a substantial rise of OPC, specifically HPV positive related as well as the change in RT delivery via IMRT.

Current controversy regarding whether the simultaneous rise of OPC and ORN was the basis of the current research by focusing on the oral and dental tissues with superimposition of their RT doses in this specific group. The current clinical debate is whether the rise of OPC and ORN is coincidental or causality. In the author's view based on the findings the rise of ORN alongside the simultaneous rise of OPC are not coincidental. This tumour group has seen a substantial increase and the within our institute totalling 42% of all cases seen for pre-RT dental assessment from 2011-2017. Add to this the favourable outcome in the HPV positive group which are the majority of OPC patients. Hence the survival pool continues to grow each year. IMRT through its multi

beams leads to widespan coverage of the jaws receiving RT. The consequence of this is the impact RT has on the non-targeted areas leading to hard and soft tissue vascular depletion. Therefore, the dentition is vulnerable to radiation caries and periodontal disease invites the risk of ORN within a compromised tissue bed.

As the management of OPC primarily remains via RT this group of patients will remain vulnerable to ORN. Though clinical trials are looking at providing lower doses of RT doses patients still receive above the 40Gy threshold and their long-term survival allows for RIF changes to continue to progress. With improving dental health the next generation of patients will present with an even better dentition than the current OPC population. Hence, the risk of ORN appears likely to stay. Of the various sub-sites for HNC patients, OPC appears to be afflicted the most and within this group specifically the HPV positive sub-cohort. These patients do not fit stereotypical HNC profile, although from a histopathological perspective their tumours are the same as other HNCs. Though distinctly different and with favourable survival outcomes, HPV positive OPC patients are managed identically to their HPV negative colleagues by both the oncology and dental sector which in part may explain the skewed leniency for why this group suffers from a heavier burden of ORN. Though strategic efforts have been employed to avoid the occurrence of ORN these have not worked for this group and their distinct difference to other HNC patients is a plausible explanation.

Though HPV positive patients have more presenting teeth at the pre-RT phase it is apparent they also have higher historic dental disease burden too. Their dentition is often heavily restored with the current generation of HPV positive OPC showing the marks of being part of the 'heavy metal' era. This complex dental presentation invites its own challenges which require long term maintenance however the added threat of a changing oral environment post-RT makes each tooth a vulnerable target for radiation caries and subsequent extraction. With this group exhibiting more teeth and long-term survival this threat continues lifelong but simultaneously there are hard and soft tissue changes as a result of radiation damage.

Previous radical preventive strategies such as mass pre-RT dental extractions have failed and are counterproductive. However, prevention remains the key to avoiding ORN. If RIF changes can be slowed, delayed or even eliminated the risk of ORN is likely to fall accordingly. Medical management in reversing radiation damage has seen some recent success. Additionally, prophylaxis in preventing ORN when dental extractions are required post-RT has also seen significant success. Extending this is the next logical step. Providing prophylaxis against RIF at the earliest possible opportunity would be highly advantageous if it works. This would allow for optimal treatment with RT while retaining teeth for maximum function. Subsequently this will

allow for continual oral intake and the continual use of the masticatory and swallowing muscles to limit trismus and dysphagia.

The century old condition of ORN remains yet to be resolved. A new era of HNC patients, novel RT delivery system and improved dental health still has not seen ORN diminish. The complication remains rife and the current work provides some valuable insight into why it continues to be seen even in OPC HPV positive patient who are not seen to fit the traditional HNC profile.

8.3 Future work

The work presented in this thesis highlights the evolving participation of the dentition, radiation delivery system and cancer sub-sites all of which contribute to developing ORN. The focus within this work was to concentrate these factors specifically on OPC. However, the same detailed assessment of the dentition and IMRT doses needs to be evaluated for various other HNC sub-sites. For the latter, once the RT doses are available an electronic matrix can be made available to all dental oncologist to allow for tailored and accurate pre-RT treatment planning. A pilot version has been created for OPC based on the current data (V Patel et al., 2019) and requires expanding with more tumour sites and improved graphics and user interface. Beyond this it would be ideal to develop a mathematical model using RT tumour dose, RT total volume and time after RT to help determine which teeth to target for vulnerability to ORN and loss of vitality.

Dental implants are a vital treatment option to achieve oral rehabilitation in HNC patients. The bone core study has provided invaluable insight regarding the RT changes in hard tissue but requires further assessment including investigation of cell cultures and having a larger cohort to examine. Understanding the cell dynamics can then allow consideration towards adapting implant surfaces to benefit the compromised bone they are housed in as well as the unique oral environment and microbiome of HNC patients.

Finally, RIF is a well-established pathophysiology of RT. For the foreseeable future RT is a mainstay treatment option and will continue to be routinely used. Hence, its effect over time upon function on HNC is a significant burden as shown by the results within these studies. If pre-RT dental extractions are to be avoided, then prevention for the need for extraction and RIF remains the answer. Currently one trial (PenVe) (ISRCTN, 2019) is assessing whether these effects can be reduced or avoided via prophylactic PVe while another study provided promising results using statins (Bourgier et al., 2019). The next phase would be to investigate these medications in a two-arm trial and obtain bone cores for histopathological assessment of RIF reduction. In addition, regenerative procedures following dental extractions in irradiated bone via local procedures such

as platelet rich fibrin and stem cell therapy remains areas in need of further assessment and research.

Supplementary Figures

N = 31		UR8	UR7	UR6	UR5	UR4	UR3	UR2	UR1		UL1	UL2	UL3	UL4	UL5	UL6	UL7	UL8
	T1N0 L	41.2	38.6	36.4	31.6	24.4	20.7	25.5	28.1		29	31.4	32.9	36.8	43.1	51.1	59.3	63.4
	T1N0 R	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
	T1N1 L	32.7	35	36.4	35.7	33.6	27.7	31	33		32.9	34.9	37.9	44.3	52	59.3	61.2	63.6
	T1N1 R	64	58.2	48.9	37.5	36.9	28.7	29.3	29.7		26.3	18.8	15.4	18.4	23.6	28.8	33.2	37.4
	T1N2 L	49.1	47.4	44.9	42.1	38.9	32.5	30.8	30.2		32.1	33.8	35.4	38.3	42.6	48.8	56.9	61.5
	T1N2 R	56.9	52.5	46.9	46	38.2	35.9	34.2	32.9		32.5	33.6	36	40.7	45.1	49.6	51.5	52.6
	T1N3 L	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
	T1N3 R	64.4	57.6	48.5	44	30.9	21.2	19.2	17.8		22.2	25.5	25.8	27.3	29.8	30.8	33.2	44.2
	T1N3 R	63.6	63.4	57.7	52	45	41.2	40.6	40.9		40.4	40.9	39.6	39.1	39.6	42.1	47.3	44
	T1N3 L	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
	T1N2 R	62.1	59.4	54.1	46.6	40.7	37.5	35.2	33.7		33.4	33.4	37.2	42.7	46.9	49	49.7	49.9
	T1N2 L	47.9	48.9	49.6	47.8	43	38.2	35.7	35.2		35.3	36.1	38.5	43.4	51.6	58.3	61.8	63
	T1N1 R	62.8	60.6	51.6	42.5	45.7	38.4	33.7	34.4		37.7	34.8	24	24.8	28.9	31.9	32.9	34.9
	T1N1 L	23.9	26.9	34.1	36	36.7	38.3	40.9	40.6		41	42.5	45.7	50.7	57.5	62.8	64.8	64
	T1N0 R	64.6	61.7	49.4	34.9	30.8	32	30.7	27.8		21.4	20.7	19.9	18.6	18.9	23.6	25.5	26.8
	T1N0 L	29.8	32.8	35.7	33.9	31.3	29.3	30.6	29.6		28.4	29.5	31.7	34.1	40	51.4	61	61.7
		LR8	LR7	LR6	LR5	LR4	LR3	LR2	LR1		LL1	LL2	LL3	LL4	LL5	LL6	LL7	LL8

65	35
55	25
45	15

Supplementary figure 1: Average radiation doses per tooth as DMean for T1 tumours with varying nodal status

		UR8	**UR7**	**UR6**	**UR5**	**UR4**	**UR3**	**UR2**	**UR1**		**UL1**	**UL2**	**UL3**	**UL4**	**UL5**	**UL6**	**UL7**	**UL8**
	T2N0 L	45.8	41.9	38.6	32.4	27.4	25	24.8	24		25.4	27.9	28.9	33.6	39.4	47.6	56.9	64
	T2N0 R	59.3	57.9	50.2	41.3	37.4	35.3	32.6	31.9		31.1	26.7	23	25.6	27.6	27.8	29.4	35.6
	T2N1 L	59.8	55.6	57.4	55	47.9	41.2	37.6	34.9		35.3	36.2	38.4	39.7	43.2	49.5	59.7	64.8
	T2N1 R	61.6	52.7	43.5	40.4	36.6	33.5	30.5	28.9		30	32	34.1	40.5	46.9	52.5	57.1	60.4
	T2N2 L	53.9	50.5	48.7	45.4	41.4	37.1	35.6	35		35.7	37.7	39.5	42.2	45.6	50.5	58	63.4
	T2N2 R	61.6	56.8	51.3	46.6	42.2	38.6	36.5	34.2		33.3	33.7	35.3	38.7	42.6	46.4	48.9	52.6
	T2N3 L	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
	T2N3 R	64.3	60.4	56.1	50.3	46.7	44.6	42.2	40.2		39.5	43.1	47.7	53.4	55.4	55.7	57.5	61.4
N = 62																		
	T2N3 R	61.7	60.8	58.6	52.5	45.7	40	36.8	36.1		36.7	39.3	33.8	51.9	57	56.8	55.9	55.7
	T2N3 L	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
	T2N2 R	63.1	61.7	57.4	51.3	47	43.3	40.1	38.6		38.1	38.4	40.1	43.8	47.9	50.2	51	51.5
	T2N2 L	51.2	49.9	49.4	47.8	44.1	40.4	38.8	38.4		38.8	39.5	41.7	46.7	52.1	57.4	61.6	63.1
	T2N1 R	64.9	62.7	55.9	47.7	39	33.4	31	30.6		32.7	34.9	38.3	45.2	49.8	53.9	56.6	59.1
	T2N1 L	49	51.5	54.5	54.5	48.8	41.6	38.1	37.9		39.1	39.7	40.6	43.7	48.4	53.3	58.9	62.5
	T2N0 R	61.3	59	52.1	43.7	39.6	37.7	36.7	37		37.2	36.8	35.6	35.7	36.8	36.5	36.1	36.7
	T2N0 L	40.6	42.4	42.3	39.1	36.5	34.9	33.8	32.1		32.4	34.4	37.3	42.7	48.5	56.4	62.2	63.5
		LR8	**LR7**	**LR6**	**LR5**	**LR4**	**LR3**	**LR2**	**LR1**		**LL1**	**LL2**	**LL3**	**LL4**	**LL5**	**LL6**	**LL7**	**LL8**

65	35
55	25
45	15

Supplementary figure 2: Average radiation doses per tooth as DMean for T2 tumours with varying nodal status

		UR8	UR7	UR6	UR5	UR4	UR3	UR2	UR1		UL1	UL2	UL3	UL4	UL5	UL6	UL7	UL8
	T3N0 L	50.5	46.6	44.6	39.3	41.2	37.5	34.9	34.6		36.4	36.3	36.7	41.6	40.4	48.5	54.7	61.2
	T3N0 R	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
	T3N1 L	60.8	52.9	51	45.1	37.6	28.5	22.9	19.5		19.2	20.1	22.7	28.9	38	49.3	54.7	61.6
	T3N1 R	64.2	64.4	63.5	63.4	60.5	55	51.9	48.9		47	47.9	50.1	54.9	56.1	55.9	52.7	55.9
	T3N2 L	52.6	48.5	46.3	42.9	38.1	34.1	32.8	32.3		32.6	34.1	35.4	38.5	43	50.7	56.5	60.9
	T3N2 R	62	59.2	54.2	50.5	46.6	42	39.1	36.8		35.8	39.3	43.9	47.8	50.3	50.8	51.6	54.6
	T3N3 L	59.5	56.1	52.6	46.4	38.3	31.7	24.4	21		19.1	19.9	23.2	28.4	38.6	49.6	59.8	63.8
	T3N3 R	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
N = 33																		
	T3N3 R	64.5	58.4	52.2	49.5	47.4	46	41	35.7		35.6	36.9	37.8	39.2	42.5	46.1	48.3	50.8
	T3N3 L	60.7	61.6	58	49	41.4	33	28.4	28		28.7	30.3	32.9	37.9	43.3	52.8	59.8	61.3
	T3N2 R	63.4	62.9	61	56.6	52	47.2	42.7	40.3		39.7	40.3	42.6	47.5	51.2	52.4	50.9	49.4
	T3N2 L	52.2	50.8	48.7	46.3	43	39.2	37	36.1		36.9	38.2	40.2	44.6	49.6	56	60.8	62.3
	T3N1 R	63.5	64.1	65	64.7	62.2	58.7	57.5	58		58.6	59.5	60.2	61.5	60.7	60	54.5	54
	T3N1 L	55.9	58	55	52.2	48.4	40	30.4	26.5		23.6	22.8	24.9	31.5	47.4	58.5	60.8	59.7
	T3N0 R	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
	T3N0 L	50.1	48.3	47.4	47.9	46.5	42.7	39.1	37		36.6	37.4	39.7	46.8	55.8	61.5	62.5	62.8
		LR8	**LR7**	**LR6**	**LR5**	**LR4**	**LR3**	**LR2**	**LR1**		**LL1**	**LL2**	**LL3**	**LL4**	**LL5**	**LL6**	**LL7**	**LL8**

65	35
55	25
45	15

Supplementary figure 3: Average radiation doses per tooth as DMean for T3 tumours with varying nodal status

		UR8	UR7	UR6	UR5	UR4	UR3	UR2	UR1		UL1	UL2	UL3	UL4	UL5	UL6	UL7	UL8
	T4N0 L	56.1	53.1	50.1	44.5	39.6	33.7	31.1	29.9		31.1	33.5	35.7	40	45.5	51.2	58.7	64.8
	T4N0 R	65.5	63.9	59.8	52.8	45.4	39	28.9	25.4		24.7	28	30.2	32.9	37.4	42.6	47.2	51.1
	T4N1 L	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
	T4N1 R	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
	T4N2 L	53.2	49.6	46.8	43.4	40	36.4	35	33.4		35.7	38.6	40.2	42.8	46	51.1	56.6	60
	T4N2 R	63.1	55.7	49.8	44.9	40.6	36.2	34.5	34.3		34.2	33.8	33.7	37	40	43.5	46.7	52.1
	T4N3 L	58.8	55.9	49.7	46.8	40.5	37.9	36.1	35.5		39.4	42.6	45.4	48.8	59.2	54.2	60.9	65.5
	T4N3 R	63.3	62.3	61.3	59.1	-	53.6	49.2	46.1		43.7	42.8	44	-	51.7	56.8	57.9	60.1
N = 34																		
	T4N3 R	63.9	63.9	63.7	61.1	-	54.5	49.9	45.6		43.8	44.9	49.4	-	52.1	50.1	48.4	43.6
	T4N3 L	59.5	59.3	58.2	56.2	51.9	47	44.1	42.9		42.8	43.4	45	50.1	56.5	60.8	64.2	65.4
	T4N2 R	65.4	65.7	62.6	57.2	52.1	48.9	46	44.5		43.5	43.8	45.4	47.8	51.5	53.9	57.6	59
	T4N2 L	55.9	54.6	53	51.6	48.1	45.9	44	43.4		43.4	44.4	46.8	50.9	56	58.8	62.1	63.2
	T4N1 R	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
	T4N1 L	52.6	54.2	60	59.8	59.3	57.8	55	52.5		50.1	50.8	53.9	58.8	62.2	63.9	64	62.3
	T4N0 R	63.5	63	59	46.7	41.4	39.7	35.6	28.9		25	23.3	23.3	25.1	28.9	30	31.9	36.1
	T4N0 L	53.4	52.8	51.2	49.5	46.2	42	37.9	35.5		34.1	34.1	35.8	40.5	47.2	55.3	60.8	63.2
		LR8	**LR7**	**LR6**	**LR5**	**LR4**	**LR3**	**LR2**	**LR1**		**LL1**	**LL2**	**LL3**	**LL4**	**LL5**	**LL6**	**LL7**	**LL8**

65	35
55	25
45	15

Supplementary figure 4: Average radiation doses per tooth as DMean for T4 tumours with varying nodal status

		UR8	UR7	UR6	UR5	UR4	UR3	UR2	UR1		UL1	UL2	UL3	UL4	UL5	UL6	UL7	UL8
	T1N0 L	41.2	38.6	36.4	31.6	24.4	20.7	25.5	28.1		29	31.4	32.9	36.8	43.1	51.1	59.3	63.4
	T1N0 R	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
	T2N0 L	45.8	41.9	38.6	32.4	27.4	25	24.8	24		25.4	27.9	28.9	33.6	39.4	47.6	56.9	64
	T2N0 R	59.3	57.9	50.2	41.3	37.4	35.3	32.6	31.9		31.1	26.7	23	25.6	27.6	27.8	29.4	35.6
	T3N0 L	50.5	46.6	44.6	39.3	41.2	37.5	34.9	34.6		36.4	36.3	36.7	41.6	40.4	48.5	54.7	61.2
	T3N0 R	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
	T4N0 L	56.1	53.1	50.1	44.5	39.6	33.7	31.1	29.9		31.1	33.5	35.7	40	45.5	51.2	58.7	64.8
	T4N0 R	65.5	63.9	59.8	52.8	45.4	39	28.9	25.4		24.7	28	30.2	32.9	37.4	42.6	47.2	51.1
N = 30																		
	T4N0 R	63.5	63	59	46.7	41.4	39.7	35.6	28.9		25	23.3	23.3	25.1	28.9	30	31.9	36.1
	T4N0 L	53.4	52.8	51.2	49.5	46.2	42	37.9	35.5		34.1	34.1	35.8	40.5	47.2	55.3	60.8	63.2
	T3N0 R	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
	T3N0 L	50.1	48.3	47.4	47.9	46.5	42.7	39.1	37		36.6	37.4	39.7	46.8	55.8	61.5	62.5	62.8
	T2N0 R	61.3	59	52.1	43.7	39.6	37.7	36.7	37		37.2	36.8	35.6	35.7	36.8	36.5	36.1	36.7
	T2N0 L	40.6	42.4	42.3	39.1	36.5	34.9	33.8	32.1		32.4	34.4	37.3	42.7	48.5	56.4	62.2	63.5
	T1N0 R	64.6	61.7	49.4	34.9	30.8	32	30.7	27.8		21.4	20.7	19.9	18.6	18.9	23.6	25.5	26.8
	T1N0 L	29.8	32.8	35.7	33.9	31.3	29.3	30.6	29.6		28.4	29.5	31.7	34.1	40	51.4	61	61.7
		LR8	**LR7**	**LR6**	**LR5**	**LR4**	**LR3**	**LR2**	**LR1**		**LL1**	**LL2**	**LL3**	**LL4**	**LL5**	**LL6**	**LL7**	**LL8**

65	35
55	25
45	15

Supplementary figure 5: Average radiation doses per tooth as DMean for N0 tumours with varying tumour size

		UR8	UR7	UR6	UR5	UR4	UR3	UR2	UR1		UL1	UL2	UL3	UL4	UL5	UL6	UL7	UL8
	T1N1 L	32.7	35	36.4	35.7	33.6	27.7	31	33		32.9	34.9	37.9	44.3	52	59.3	61.2	63.6
	T1N1 R	64	58.2	48.9	37.5	36.9	28.7	29.3	29.7		26.3	18.8	15.4	18.4	23.6	28.8	33.2	37.4
	T2N1 L	59.8	55.6	57.4	55	47.9	41.2	37.6	34.9		35.3	36.2	38.4	39.7	43.2	49.5	59.7	64.8
	T2N1 R	61.6	52.7	43.5	40.4	36.6	33.5	30.5	28.9		30	32	34.1	40.5	46.9	52.5	57.1	60.4
	T3N1 L	60.8	52.9	51	45.1	37.6	28.5	22.9	19.5		19.2	20.1	22.7	28.9	38	49.3	54.7	61.6
	T3N1 R	64.2	64.4	63.5	63.4	60.5	55	51.9	48.9		47	47.9	50.1	54.9	56.1	55.9	52.7	55.9
	T4N1 L	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
	T4N1 R	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
N = 14																		
	T4N1 R	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
	T4N1 L	52.6	54.2	60	59.8	59.3	57.8	55	52.5		50.1	50.8	53.9	58.8	62.2	63.9	64	62.3
	T3N1 R	63.5	64.1	65	64.7	62.2	58.7	57.5	58		58.6	59.5	60.2	61.5	60.7	60	54.5	54
	T3N1 L	55.9	58	55	52.2	48.4	40	30.4	26.5		23.6	22.8	24.9	31.5	47.4	58.5	60.8	59.7
	T2N1 R	64.9	62.7	55.9	47.7	39	33.4	31	30.6		32.7	34.9	38.3	45.2	49.8	53.9	56.6	59.1
	T2N1 L	49	51.5	54.5	54.5	48.8	41.6	38.1	37.9		39.1	39.7	40.6	43.7	48.4	53.3	58.9	62.5
	T1N1 R	62.8	60.6	51.6	42.5	45.7	38.4	33.7	34.4		37.7	34.8	24	24.8	28.9	31.9	32.9	34.9
	T1N1 L	23.9	26.9	34.1	36	36.7	38.3	40.9	40.6		41	42.5	45.7	50.7	57.5	62.8	64.8	64
		LR8	LR7	LR6	LR5	LR4	LR3	LR2	LR1		LL1	LL2	LL3	LL4	LL5	LL6	LL7	LL8

65	35
55	25
45	15

Supplementary figure 6: Average radiation doses per tooth as DMean for N1 tumours with varying tumour size

N = 107		UR8	UR7	UR6	UR5	UR4	UR3	UR2	UR1		UL1	UL2	UL3	UL4	UL5	UL6	UL7	UL8
	T1N2 L	49.1	47.4	44.9	42.1	38.9	32.5	30.8	30.2		32.1	33.8	35.4	38.3	42.6	48.8	56.9	61.5
	T1N2 R	56.9	52.5	46.9	46	38.2	35.9	34.2	32.9		32.5	33.6	36	40.7	45.1	49.6	51.5	52.6
	T2N2 L	53.9	50.5	48.7	45.4	41.4	37.1	35.6	35		35.7	37.7	39.5	42.2	45.6	50.5	58	63.4
	T2N2 R	61.6	56.8	51.3	46.6	42.2	38.6	36.5	34.2		33.3	33.7	35.3	38.7	42.6	46.4	48.9	52.6
	T3N2 L	52.6	48.5	46.3	42.9	38.1	34.1	32.8	32.3		32.6	34.1	35.4	38.5	43	50.7	56.5	60.9
	T3N2 R	62	59.2	54.2	50.5	46.6	42	39.1	36.8		35.8	39.3	43.9	47.8	50.3	50.8	51.6	54.6
	T4N2 L	53.2	49.6	46.8	43.4	40	36.4	35	33.4		35.7	38.6	40.2	42.8	46	51.1	56.6	60
	T4N2 R	63.1	55.7	49.8	44.9	40.6	36.2	34.5	34.3		34.2	33.8	33.7	37	40	43.5	46.7	52.1
	T4N2 R	65.4	65.7	62.6	57.2	52.1	48.9	46	44.5		43.5	43.8	45.4	47.8	51.5	53.9	57.6	59
	T4N2 L	55.9	54.6	53	51.6	48.1	45.9	44	43.4		43.4	44.4	46.8	50.9	56	58.8	62.1	63.2
	T3N2 R	63.4	62.9	61	56.6	52	47.2	42.7	40.3		39.7	40.3	42.6	47.5	51.2	52.4	50.9	49.4
	T3N2 L	52.2	50.8	48.7	46.3	43	39.2	37	36.1		36.9	38.2	40.2	44.6	49.6	56	60.8	62.3
	T2N2 R	63.1	61.7	57.4	51.3	47	43.3	40.1	38.6		38.1	38.4	40.1	43.8	47.9	50.2	51	51.5
	T2N2 L	51.2	49.9	49.4	47.8	44.1	40.4	38.8	38.4		38.8	39.5	41.7	46.7	52.1	57.4	61.6	63.1
	T1N2 R	62.1	59.4	54.1	46.6	40.7	37.5	35.2	33.7		33.4	33.4	37.2	42.7	46.9	49	49.7	49.9
	T1N2 L	47.9	48.9	49.6	47.8	43	38.2	35.7	35.2		35.3	36.1	38.5	43.4	51.6	58.3	61.8	63
		LR8	LR7	LR6	LR5	LR4	LR3	LR2	LR1		LL1	LL2	LL3	LL4	LL5	LL6	LL7	LL8

65	35
55	25
45	15

Supplementary figure 7: Average radiation doses per tooth as DMean for N2 tumours with varying tumour size

N = 9		UR8	UR7	UR6	UR5	UR4	UR3	UR2	UR1		UL1	UL2	UL3	UL4	UL5	UL6	UL7	UL8
	T1N3 L	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
	T1N3 R	64.4	57.6	48.5	44	30.9	21.2	19.2	17.8		22.2	25.5	25.8	27.3	29.8	30.8	33.2	44.2
	T2N3 L	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
	T2N3 R	64.3	60.4	56.1	50.3	46.7	44.6	42.2	40.2		39.5	43.1	47.7	53.4	55.4	55.7	57.5	61.4
	T3N3 L	59.5	56.1	52.6	46.4	38.3	31.7	24.4	21		19.1	19.9	23.2	28.4	38.6	49.6	59.8	63.8
	T3N3 R	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
	T4N3 L	58.8	55.9	49.7	46.8	40.5	37.9	36.1	35.5		39.4	42.6	45.4	48.8	59.2	54.2	60.9	65.5
	T4N3 R	63.3	62.3	61.3	59.1	-	53.6	49.2	46.1		43.7	42.8	44	-	51.7	56.8	57.9	60.1
	T4N3 R	63.9	63.9	63.7	61.1	-	54.5	49.9	45.6		43.8	44.9	49.4	-	52.1	50.1	48.4	43.6
	T4N3 L	59.5	59.3	58.2	56.2	51.9	47	44.1	42.9		42.8	43.4	45	50.1	56.5	60.8	64.2	65.4
	T3N3 R	64.5	58.4	52.2	49.5	47.4	46	41	35.7		35.6	36.9	37.8	39.2	42.5	46.1	48.3	50.8
	T3N3 L	60.7	61.6	58	49	41.4	33	28.4	28		28.7	30.3	32.9	37.9	43.3	52.8	59.8	61.3
	T2N3 R	61.7	60.8	58.6	52.5	45.7	40	36.8	36.1		36.7	39.3	33.8	51.9	57	56.8	55.9	55.7
	T2N3 L	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
	T1N3 R	63.6	63.4	57.7	52	45	41.2	40.6	40.9		40.4	40.9	39.6	39.1	39.6	42.1	47.3	44
	T1N3 L	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
		LR8	LR7	LR6	LR5	LR4	LR3	LR2	LR1		LL1	LL2	LL3	LL4	LL5	LL6	LL7	LL8

65	35
55	25
45	15

Supplementary figure 8: Average radiation doses per tooth as DMean for N3 tumours with varying tumour size

	Rosenthal	Patel
Mandible - Anteriors	31.5	41.5 – 37.8
Mandible - Premolars	36.1	49.0 – 44.3
Mandible - Molars	47.7	56.5 – 52.9
Maxilla - Anterior	24.6	36.3 – 33.0
Maxilla - Premolars	24.6	44.8 – 39.8
Maxilla - Molars	30.1	57.5 – 48.4

Supplementary figure 9: Rosenthal et al (15 oropharyngeal cancer patients) doses versus doses identified from this study

	BoT	Tonsil	Patel
Mandible - Anteriors	33.6	34.9	41.5 – 37.8
Mandible - Premolars	40.4	42.5	49.0 – 44.3
Mandible – 1st Molar	46.7	52.3	53.1 – 52.9
Mandible - 2nd Molar	51.0	48.3	55.6 – 55.5
Mandible – 3rd Molar	60.3	50.0	56.5
Maxilla - Anteriors	27.9	38.9	36.3 – 33.0
Maxilla - Premolars	35.7	41.8	44.8 – 39.8
Maxilla – 1st Molar	41.4	48.8	48.6 – 48.4
Maxilla - 2nd Molar	43.8	47.6	53.2 – 52.4
Maxilla – 3rd Molar	51.0	50.0	57.5 – 56.4

Supplementary figure 10: Bak et al doses versus doses identified from this study

	T1-2/N2-3	Patel	T3-4/N2-3	Patel	T1-4N0	Patel
Ipsilateral Molars	47.6	T1N2: 63.0 - 54.1	60.1	T3N2: 63.4 – 56.0	58.1	T1N0: 64.6 – 49.4
		T1N3: 63.6 – 57.7		T3N3: 64.5 – 52.2		T2N0: 63.5 – 52.1
		T2N2: 63.1 – 57.4		T4N2: 65.4 – 58.8		T3N0: 62.8 – 61.5
		T2N3: 61.7 – 58.6		T4N3: 65.4 – 60.8		T4N0: 63.5 – 55.3
Ipsilateral Premolars	32.6	T1N2: 51.6 – 43.0	53.3	T3N2: 56.6 – 44.6	40.2	T1N0: 40.0 – 30.8
		T1N3: 52.0 – 39.1		T3N3: 49.5 – 37.9		T2N0: 48.5 – 39.7
		T2N2: 52.1 – 47.0		T4N2: 57.2 – 50.9		T3N0: 55.8 – 46.8
		T2N3: 52.5 – 45.7		T4N3: 61.1 – 50.1		T4N0: 47.2 – 40.5
Contralateral Molars	49.4	T1N2: 49.9 – 47.9	56.0	T3N2: 52.2 – 49.4	49.4	T1N0: 35.7 – 23.6
		T1N3: 47.3 – 42.1		T3N3: 61.6 – 46.1		T2N0: 42.3 – 36.7
		T2N2: 51.5 – 49.4		T4N2: 59.0 – 53.0		T3N0: 50.1 – 47.4
		T2N3: 56.8 – 55.9		T4N3: 59.5 – 43.6		T4N0: 53.4 – 30.0
Contralateral Premolars	27.4	T1N2: 47.8 – 42.7	51.1	T3N2: 52.4 – 43.0	35.5	T1N0: 31.3 – 18.6
		T1N3: 39.6 – 39.1		T3N3: 49.0 – 39.2		T2N0: 39.1 – 35.7
		T2N2: 47.9 – 43.8		T4N2: 51.6 – 47.8		T3N0: 47.9 – 46.5
		T2N3: 57.0 - 519		T4N3: 56.2 – 51.9		T4N0: 49.5 – 25.1
Anteriors	23.8	T1N2: 38.5 – 33.4	47.3	T3N2: 47.2 – 36.1	26.1	T1N0: 32.0 – 20.7
		T1N3: 41.2 – 39.6		T3N3: 46.0 – 28.0		T2N0: 37.7 – 32.1
		T2N2: 43.3 – 38.4		T4N2: 48.9 – 43.4		T3N0: 42.7 – 36.6
		T2N3: 40.0 – 33.8		T4N3: 54.5 – 42.8		T4N0: 42.0 – 23.3

Supplementary figure 11: Hansen et al doses (28 patients) vs doses identified from this study

	Parahyba (Dmax)	Patel (Dmax)	Parahyba (Dmean)	Patel (Dmean)
Upper right quadrant	35.7	62.7 – 46.8	27.4	56.4 – 40.0
Upper anteriors	26.1	42.8 – 42.2	16.6	36.3 – 36.1
Upper left quadrant	35.2	63.6 – 46.4	26.4	57.5 – 39.8
Lower left quadrant	44.3	63.1 – 50.9	35.3	56.5 – 44.3
Lower anteriors	35.5	47.8 – 46.5	25.4	41.5 – 40.2
Lower right quadrants	43.3	63.0 – 51.3	34.5	56.5 – 44.9

Supplementary figure 12: Parahyba et al doses versus doses identified from this study

References

AAOMS. (2014). Medication-Related Osteonecrosis of the Jaw—2014 Update. Retrieved from https://www.aaoms.org/docs/govt_affairs/advocacy_white_papers/mronj_position_paper.pdf

Aarup-Kristensen, S., Hansen, C. R., Forner, L., Brink, C., Eriksen, J. G., & Johansen, J. (2019). Osteoradionecrosis of the mandible after radiotherapy for head and neck cancer: risk factors and dose-volume correlations. *Acta Oncol, 58*(10), 1373-1377. doi:10.1080/0284186x.2019.1643037

Adelstein, D. J., Ridge, J. A., Gillison, M. L., Chaturvedi, A. K., D'Souza, G., Gravitt, P. E., . . . Ullmann, C. D. (2009). Head and neck squamous cell cancer and the human papillomavirus: summary of a National Cancer Institute State of the Science Meeting, November 9-10, 2008, Washington, D.C. *Head Neck, 31*(11), 1393-1422. doi:10.1002/hed.21269

ADHS. (2009). Adult Dental Health Survey 2009 - Summary report and thematic series. Retrieved from https://digital.nhs.uk/data-and-information/publications/statistical/adult-dental-health-survey/adult-dental-health-survey-2009-summary-report-and-thematic-series

AHNS. (2014). TNM Staging of Head and Neck Cancer and Neck Dissection Classification. Retrieved from https://www.entnet.org/sites/default/files/NeckDissection_QuickRefGuide_highresFINAL.pdf

Alessandro, S. G., Antonino, C., & Messina, P. (2009). Anatomical evaluation of oral microcirculation: capillary characteristics associated with sex or age group. *Annals of Anatomy-Anatomischer Anzeiger, 191*(4), 371-378.

Anantharaman, D., Gheit, T., Waterboer, T., Abedi-Ardekani, B., Carreira, C., McKay-Chopin, S., . . . Brennan, P. (2013). Human papillomavirus infections and upper aero-digestive tract cancers: the ARCAGE study. *J Natl Cancer Inst, 105*(8), 536-545. doi:10.1093/jnci/djt053

Ang, K. K., Harris, J., Wheeler, R., Weber, R., Rosenthal, D. I., Nguyen-Tan, P. F., . . . Gillison, M. L. (2010). Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med, 363*(1), 24-35. doi:10.1056/NEJMoa0912217

Ang, K. K., & Sturgis, E. M. (2012). Human papillomavirus as a marker of the natural history and response to therapy of head and neck squamous cell carcinoma. *Semin Radiat Oncol, 22*(2), 128-142. doi:10.1016/j.semradonc.2011.12.004

Anitei, M. G., Zeitoun, G., Mlecnik, B., Marliot, F., Haicheur, N., Todosi, A. M., . . . Pagès, F. (2014). Prognostic and predictive values of the immunoscore in patients with rectal cancer. *Clin Cancer Res, 20*(7), 1891-1899. doi:10.1158/1078-0432.Ccr-13-2830

Arganda-Carreras, I., Kaynig, V., Rueden, C., Eliceiri, K. W., Schindelin, J., Cardona, A., & Sebastian Seung, H. (2017). Trainable Weka Segmentation: a machine learning tool for microscopy pixel classification. *Bioinformatics, 33*(15), 2424-2426.

Auluck, A., Hislop, G., Bajdik, C., Poh, C., Zhang, L., & Rosin, M. (2010). Trends in oropharyngeal and oral cavity cancer incidence of human papillomavirus (HPV)-related and HPV-unrelated sites in a multicultural population: the British Columbia experience. *Cancer, 116*(11), 2635-2644. doi:10.1002/cncr.25087

Axelsson, P., Paulartder, J., & Lindhe, J. (1998). Relationship between smoking and dental status in 35-, 50-, 65-, and 75-year-old individuals. *Journal of clinical periodontology, 25*(4), 297-305.

Badoual, C., Hans, S., Merillon, N., Van Ryswick, C., Ravel, P., Benhamouda, N., . . . Tartour, E. (2013). PD-1-expressing tumor-infiltrating T cells are a favorable prognostic biomarker in HPV-associated head and neck cancer. *Cancer Res, 73*(1), 128-138. doi:10.1158/0008-5472.Can-12-2606

BAHNO. (2016). Head and Neck Cancer:

United Kingdom National Multidisciplinary Guidelines Retrieved from https://www.bahno.org.uk/_userfiles/pages/files/ukheadandcancerguidelines2016.pdf

Bak, S. Y., Qi, X. S., Kelly, J. A., Alexander, S., Chung, Y., Gyurdzhyan, S., . . . Lee, S. P. (2016). Dosimetric distribution to tooth-bearing areas in intensity-modulated radiation therapy for head and neck cancer: a pilot study. *Oral Surg Oral Med Oral Pathol Oral Radiol, 121*(1), 43-48. doi:10.1016/j.oooo.2015.10.007

Baker, D. G., & Krochak, R. J. (1989). The response of the microvascular system to radiation: a review. *Cancer investigation, 7*(3), 287-294.

Balermpas, P., Rödel, F., Rödel, C., Krause, M., Linge, A., Lohaus, F., . . . Fokas, E. (2016). CD8+ tumour-infiltrating lymphocytes in relation to HPV status and clinical outcome in patients with head and neck cancer after postoperative chemoradiotherapy: A multicentre study of the German cancer consortium radiation oncology group (DKTK-ROG). *International journal of cancer, 138*(1), 171-181. doi:10.1002/ijc.29683

Barnett, G. C., West, C. M. L., Dunning, A. M., Elliott, R. M., Coles, C. E., Pharoah, P. D. P., & Burnet, N. G. (2009). Normal tissue reactions to radiotherapy: towards tailoring treatment dose by genotype. *Nature reviews. Cancer, 9*(2), 134-142. doi:10.1038/nrc2587

Baskar, R., Lee, K. A., Yeo, R., & Yeoh, K. W. (2012). Cancer and radiation therapy: current advances and future directions. *Int J Med Sci, 9*(3), 193-199. doi:10.7150/ijms.3635

Bastos, A. P. (2020). *Real time optical vascular imaging for the diagnosis of oral diseases.* (PhD). King's College London,

Bastos, P., & Cook, R. (2016). Real Time Optical Vascular Imaging: A Potential Technique for the Diagnosis of Mucosal Disease Including Early Oral Cancer. *Prim Dent J, 5*(1), 86-91.

Bastos, P., Patel, V., Festy, F., Hosny, N., & Cook, R. J. (2017). In-vivo imaging of the microvasculature of the soft tissue margins of osteonecrotic jaw lesions. *Br Dent J, 223*(9), 699-705. doi:10.1038/sj.bdj.2017.888

Bedwinek, J. M., Shukovsky, L. J., Fletcher, G. H., & Daley, T. (1976). Osteonecrosis in patients treated with definitive radiotherapy for squamous cell carcinomas of the oral cavity and naso-and oropharynx. *Radiology, 119*(3), 665-667.

Beech, N., Porceddu, S., & Batstone, M. D. (2016). Preradiotherapy dental extractions and health-related quality of life. *Oral surgery, oral medicine, oral pathology and oral radiology, 122*(6), 672-679.

Beech, N. M., Porceddu, S., & Batstone, M. D. (2017). Radiotherapy-associated dental extractions and osteoradionecrosis. *Head & neck, 39*(1), 128-132.

Ben-David, M. A., Diamante, M., Radawski, J. D., Vineberg, K. A., Stroup, C., Murdoch-Kinch, C.-A., . . . Eisbruch, A. (2007). Lack of osteoradionecrosis of the mandible after intensity-modulated radiotherapy for head and neck cancer: likely contributions of both dental care and improved dose distributions. *International Journal of Radiation Oncology* Biology* Physics, 68*(2), 396-402.

Benjamini, Y., Krieger, A. M., & Yekutieli, D. (2006). Adaptive linear step-up procedures that control the false discovery rate. *Biometrika, 93*(3), 491-507.

Bennett, M. H., Feldmeier, J., Hampson, N. B., Smee, R., & Milross, C. (2016). Hyperbaric oxygen therapy for late radiation tissue injury. *Cochrane Database Syst Rev, 4*(4), Cd005005. doi:10.1002/14651858.CD005005.pub4

Berman, B., & Duncan, M. (1990). Pentoxifylline inhibits the proliferation of human fibroblasts derived from keloid, scleroderma and morphoea skin and their production of collagen, glycosaminoglycans and fibronectin. *British Journal of Dermatology, 123*(3), 339-346.

Beumer III, J., Silverman Jr, S., & Benak Jr, S. B. (1972). Hard and soft tissue necroses following radiation therapy for oral cancer. *The Journal of prosthetic dentistry, 27*(6), 640-644.

Bird, T., Barrington, S., Thavaraj, S., Jeannon, J. P., Lyons, A., Oakley, R., . . . Guerrero Urbano, T. (2016). (18)F-FDG PET/CT to assess response and guide risk-stratified follow-up after chemoradiotherapy for oropharyngeal squamous cell carcinoma. *Eur J Nucl Med Mol Imaging, 43*(7), 1239-1247. doi:10.1007/s00259-015-3290-4

Blanchard, P., Baujat, B., Holostenco, V., Bourredjem, A., Baey, C., Bourhis, J., & Pignon, J. P. (2011). Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): a comprehensive analysis by tumour site. *Radiother Oncol, 100*(1), 33-40. doi:10.1016/j.radonc.2011.05.036

Bléry, P., Espitalier, F., Hays, A., Crauste, E., Demarquay, C., Pilet, P., . . . Benderitter, M. (2015). Development of mandibular osteoradionecrosis in rats: importance of dental extraction. *Journal of Cranio-Maxillofacial Surgery, 43*(9), 1829-1836.

Blomberg, M., Nielsen, A., Munk, C., & Kjaer, S. K. (2011). Trends in head and neck cancer incidence in Denmark, 1978-2007: focus on human papillomavirus associated sites. *International journal of cancer, 129*(3), 733-741. doi:10.1002/ijc.25699

Blot, W. J., Devesa, S. S., McLaughlin, J. K., & Fraumeni, J. F., Jr. (1994). Oral and pharyngeal cancers. *Cancer Surv, 19-20*, 23-42.

BMA. (2008). Alcohol misuse: tackling the
UK epidemic. Retrieved from http://www.dldocs.stir.ac.uk/documents/Alcoholmisuse.pdf

Bodard, A.-G., Debbache, S., Langonnet, S., Laffay, F., & Fleury, B. (2013). A model of mandibular irradiation in the rabbit: preliminary results. *Bulletin du Groupement International pour la Recherche Scientifique en Stomatologie et Odontologie, 52*(1), 17-22.

Boscolo-Rizzo, P., Del Mistro, A., Bussu, F., Lupato, V., Baboci, L., Almadori, G., . . . Paludetti, G. (2013). New insights into human papillomavirus-associated head and neck squamous cell carcinoma. *Acta Otorhinolaryngologica Italica, 33*(2), 77.

Bourgier, C., Auperin, A., Rivera, S., Boisselier, P., Petit, B., Lang, P., . . . Vozenin, M. C. (2019). Pravastatin Reverses Established Radiation-Induced Cutaneous and Subcutaneous Fibrosis in Patients With Head and Neck Cancer: Results of the Biology-Driven Phase 2 Clinical Trial Pravacur. *Int J Radiat Oncol Biol Phys, 104*(2), 365-373. doi:10.1016/j.ijrobp.2019.02.024

Braakhuis, B. J., Visser, O., & Leemans, C. R. (2009). Oral and oropharyngeal cancer in The Netherlands between 1989 and 2006: Increasing incidence, but not in young adults. *Oral oncology, 45*(9), e85-e89.

Bras, J., De Jonge, H., & Van Merkesteyn, J. (1990). Osteoradionecrosis of the mandible: pathogenesis. *American journal of otolaryngology, 11*(4), 244-250.

Bucci, M. K., Bevan, A., & Roach, M., 3rd. (2005). Advances in radiation therapy: conventional to 3D, to IMRT, to 4D, and beyond. *CA Cancer J Clin, 55*(2), 117-134. doi:10.3322/canjclin.55.2.117

Calais, G., Alfonsi, M., Bardet, E., Sire, C., Germain, T., Bergerot, P., . . . Bertrand, P. (1999). Randomized trial of radiation therapy versus concomitant chemotherapy and

radiation therapy for advanced-stage oropharynx carcinoma. *J Natl Cancer Inst, 91*(24), 2081-2086. doi:10.1093/jnci/91.24.2081
Caparrotti, F., Huang, S. H., Lu, L., Bratman, S. V., Ringash, J., Bayley, A., . . . Waldron, J. (2017). Osteoradionecrosis of the mandible in patients with oropharyngeal carcinoma treated with intensity-modulated radiotherapy. *Cancer, 123*(19), 3691-3700.
CDC. (2013). Vital signs: current cigarette smoking among adults aged ≥18 years with mental illness - United States, 2009-2011. *MMWR Morb Mortal Wkly Rep, 62*(5), 81-87.
CDC. (2019). Retrieved from https://www.cdc.gov/cancer/hpv/statistics/cases.htm
CGARN. (2012). Comprehensive genomic characterization of squamous cell lung cancers. *Nature, 489*(7417), 519-525. doi:10.1038/nature11404
Chang, D. T., Sandow, P. R., Morris, C. G., Hollander, R., Scarborough, L., Amdur, R. J., & Mendenhall, W. M. (2007). Do pre-irradiation dental extractions reduce the risk of osteoradionecrosis of the mandible? *Head & Neck: Journal for the Sciences and Specialties of the Head and Neck, 29*(6), 528-536.
Chao, K. S., Ozyigit, G., Blanco, A. I., Thorstad, W. L., Deasy, J. O., Haughey, B. H., . . . Sessions, D. G. (2004). Intensity-modulated radiation therapy for oropharyngeal carcinoma: impact of tumor volume. *Int J Radiat Oncol Biol Phys, 59*(1), 43-50. doi:10.1016/j.ijrobp.2003.08.004
Chaturvedi, A. K. (2012). Epidemiology and clinical aspects of HPV in head and neck cancers. *Head Neck Pathol, 6 Suppl 1*(Suppl 1), S16-24. doi:10.1007/s12105-012-0377-0
Chaturvedi, A. K., Anderson, W. F., Lortet-Tieulent, J., Curado, M. P., Ferlay, J., Franceschi, S., . . . Gillison, M. L. (2013). Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. *J Clin Oncol, 31*(36), 4550-4559. doi:10.1200/jco.2013.50.3870
Chaturvedi, A. K., Engels, E. A., Anderson, W. F., & Gillison, M. L. (2008). Incidence trends for human papillomavirus-related and -unrelated oral squamous cell carcinomas in the United States. *J Clin Oncol, 26*(4), 612-619. doi:10.1200/jco.2007.14.1713
Chaturvedi, A. K., Engels, E. A., Pfeiffer, R. M., Hernandez, B. Y., Xiao, W., Kim, E., . . . Gillison, M. L. (2011). Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *J Clin Oncol, 29*(32), 4294-4301. doi:10.1200/jco.2011.36.4596
Chen, J. A., Wang, C. C., Wong, Y. K., Wang, C. P., Jiang, R. S., Lin, J. C., . . . Liu, S. A. (2016). Osteoradionecrosis of mandible bone in patients with oral cancer—associated factors and treatment outcomes. *Head & neck, 38*(5), 762-768.
Cheng, S.-J., Lee, J.-J., Ting, L.-L., Tseng, I.-Y., Chang, H.-H., Chen, H.-M., . . . Kok, S.-H. (2006). A clinical staging system and treatment guidelines for maxillary osteoradionecrosis in irradiated nasopharyngeal carcinoma patients. *International Journal of Radiation Oncology* Biology* Physics, 64*(1), 90-97.
ClinicalTrials.gov. (2019). Post-operative Adjuvant Treatment for HPV-positive Tumours (PATHOS) Retrieved from https://clinicaltrials.gov/ct2/show/NCT02215265
Clough, S., Burke, M., Daly, B., & Scambler, S. (2018). The impact of pre-radiotherapy dental extractions on head and neck cancer patients: a qualitative study. *Br Dent J, 225*(1), 28-32. doi:10.1038/sj.bdj.2018.442
Coffin, F. (1983). The incidence and management of osteoradionecrosis of the jaws following head and neck radiotherapy. *Br J Radiol, 56*(671), 851-857. doi:10.1259/0007-1285-56-671-851
Cooper, J. (2003). *Radiation oncology: rationale, technique, results*: Mosby.

Critchlow, S., Morgan, C., & Leung, T. (2014). The oral health status of pre-treatment head and neck cancer patients. *British dental journal, 216*(1), E1.

CRUK. (2018). Mouth and oropharyngeal cancer. Retrieved from https://www.cancerresearchuk.org/about-cancer/mouth-cancer/stages-types-grades/types-grades

Curi, M. M., Cardoso, C. L., De Lima, H. G., Kowalski, L. P., & Martins, M. D. (2016). Histopathologic and histomorphometric analysis of irradiation injury in bone and the surrounding soft tissues of the jaws. *Journal of oral and maxillofacial surgery, 74*(1), 190-199.

D'Souza, G., Agrawal, Y., Halpern, J., Bodison, S., & Gillison, M. L. (2009). Oral sexual behaviors associated with prevalent oral human papillomavirus infection. *J Infect Dis, 199*(9), 1263-1269. doi:10.1086/597755

D'Souza, G., Kreimer, A. R., Viscidi, R., Pawlita, M., Fakhry, C., Koch, W. M., . . . Gillison, M. L. (2007). Case-control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med, 356*(19), 1944-1956. doi:10.1056/NEJMoa065497

Dahlin, A. M., Henriksson, M. L., Van Guelpen, B., Stenling, R., Oberg, A., Rutegård, J., & Palmqvist, R. (2011). Colorectal cancer prognosis depends on T-cell infiltration and molecular characteristics of the tumor. *Mod Pathol, 24*(5), 671-682. doi:10.1038/modpathol.2010.234

DAHNO. (2014). National Head and Neck Cancer Audit 2014. Retrieved from https://files.digital.nhs.uk/publicationimport/pub18xxx/pub18081/clin-audi-supp-prog-head-neck-dahn-13-14.pdf

Daly, T. E., Drane, J. B., & MacComb, W. S. (1972). Management of problems of the teeth and jaw in patients undergoing irradiation. *The American Journal of Surgery, 124*(4), 539-542.

Dambrain, R. (1993). [The pathogenesis of osteoradionecrosis]. *Rev Stomatol Chir Maxillofac, 94*(3), 140-147.

Davoudi, B., Morrison, M., Bizheva, K., Yang, V. X., Dinniwell, R., Levin, W., & Vitkin, I. A. (2013). Optical coherence tomography platform for microvascular imaging and quantification: initial experience in late oral radiation toxicity patients. *J Biomed Opt, 18*(7), 76008. doi:10.1117/1.Jbo.18.7.076008

De Backer, D., Hollenberg, S., Boerma, C., Goedhart, P., Büchele, G., Ospina-Tascon, G., . . . Ince, C. (2007). How to evaluate the microcirculation: report of a round table conference. *Critical care (London, England), 11*(5), R101-R101. doi:10.1186/cc6118

de Barros da Cunha, S. R., Fonseca, F. P., Ramos, P., Haddad, C. M. K., Fregnani, E. R., & Aranha, A. C. C. (2017). Effects of different radiation doses on the microhardness, superficial morphology, and mineral components of human enamel. *Arch Oral Biol, 80*, 130-135. doi:10.1016/j.archoralbio.2017.04.007

De Felice, F., Thomas, C., Patel, V., Connor, S., Michaelidou, A., Sproat, C., . . . McGurk, M. (2016). Osteoradionecrosis following treatment for head and neck cancer and the effect of radiotherapy dosimetry: the Guy's and St Thomas' Head and Neck Cancer Unit experience. *Oral surgery, oral medicine, oral pathology and oral radiology, 122*(1), 28-34.

de Martel, C., Ferlay, J., Franceschi, S., Vignat, J., Bray, F., Forman, D., & Plummer, M. (2012). Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. *Lancet Oncol, 13*(6), 607-615. doi:10.1016/s1470-2045(12)70137-7

de Villiers, E. M. (2013). Cross-roads in the classification of papillomaviruses. *Virology, 445*(1-2), 2-10. doi:10.1016/j.virol.2013.04.023

Dekker, H., Bravenboer, N., van Dijk, D., Bloemena, E., Rietveld, D. H. F., Ten Bruggenkate, C. M., & Schulten, E. (2018). The irradiated human mandible: A quantitative study on bone vascularity. *Oral Oncol, 87*, 126-130. doi:10.1016/j.oraloncology.2018.10.030

Delanian, S., Chatel, C., Porcher, R., Depondt, J., & Lefaix, J. L. (2011). Complete restoration of refractory mandibular osteoradionecrosis by prolonged treatment with a pentoxifylline-tocopherol-clodronate combination (PENTOCLO): a phase II trial. *Int J Radiat Oncol Biol Phys, 80*(3), 832-839. doi:10.1016/j.ijrobp.2010.03.029

Delanian, S., & Lefaix, J.-L. (2004). The radiation-induced fibroatrophic process: therapeutic perspective via the antioxidant pathway. *Radiotherapy and Oncology, 73*(2), 119-131.

Delanian, S., & Lefaix, J.-L. (2007). *Current management for late normal tissue injury: radiation-induced fibrosis and necrosis.* Paper presented at the Seminars in radiation oncology.

Delanian, S., & Lefaix, J. L. (2002). Complete healing of severe osteoradionecrosis with treatment combining pentoxifylline, tocopherol and clodronate. *Br J Radiol, 75*(893), 467-469. doi:10.1259/bjr.75.893.750467

Delanian, S., Porcher, R., Balla-Mekias, S., & Lefaix, J. L. (2003). Randomized, placebo-controlled trial of combined pentoxifylline and tocopherol for regression of superficial radiation-induced fibrosis. *J Clin Oncol, 21*(13), 2545-2550. doi:10.1200/jco.2003.06.064

Denham, J. W., & Hauer-Jensen, M. (2002). The radiotherapeutic injury--a complex 'wound'. *Radiother Oncol, 63*(2), 129-145. doi:10.1016/s0167-8140(02)00060-9

Denis, F., Garaud, P., Bardet, E., Alfonsi, M., Sire, C., Germain, T., . . . Calais, G. (2004). Final results of the 94-01 French Head and Neck Oncology and Radiotherapy Group randomized trial comparing radiotherapy alone with concomitant radiochemotherapy in advanced-stage oropharynx carcinoma. *J Clin Oncol, 22*(1), 69-76. doi:10.1200/jco.2004.08.021

Denis, F., Garaud, P., Bardet, E., Alfonsi, M., Sire, C., Germain, T., . . . Calais, G. (2003). Late toxicity results of the GORTEC 94-01 randomized trial comparing radiotherapy with concomitant radiochemotherapy for advanced-stage oropharynx carcinoma: comparison of LENT/SOMA, RTOG/EORTC, and NCI-CTC scoring systems. *Int J Radiat Oncol Biol Phys, 55*(1), 93-98. doi:10.1016/s0360-3016(02)03819-1

Denkert, C., Loibl, S., Noske, A., Roller, M., Müller, B. M., Komor, M., . . . von Minckwitz, G. (2010). Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. *J Clin Oncol, 28*(1), 105-113. doi:10.1200/jco.2009.23.7370

Deshpande, S. S., Donneys, A., Farberg, A. S., Tchanque-Fossuo, C. N., Felice, P. A., & Buchman, S. R. (2014). Quantification and characterization of radiation-induced changes to mandibular vascularity using micro-computed tomography. *Ann Plast Surg, 72*(1), 100-103. doi:10.1097/SAP.0b013e318255a57d

Deshpande, S. S., Donneys, A., Kang, S. Y., Page, E. E., Felice, P. A., Kiryakoza, L., . . . Buchman, S. R. (2014). Vascular analysis as a proxy for mechanostransduction response in an isogenic, irradiated murine model of mandibular distraction osteogenesis. *Microvascular research, 95*, 143-148.

Drinkaware. (2019). Retrieved from https://www.drinkaware.co.uk/alcohol-facts/alcoholic-drinks-units/latest-uk-alcohol-unit-guidance/

Duarte, V. M., Liu, Y. F., Rafizadeh, S., Tajima, T., Nabili, V., & Wang, M. B. (2014). Comparison of dental health of patients with head and neck cancer receiving IMRT vs conventional radiation. *Otolaryngology--Head and Neck Surgery, 150*(1), 81-86.

Ducray, F., de Reyniès, A., Chinot, O., Idbaih, A., Figarella-Branger, D., Colin, C., . . . Berger, F. (2010). An ANOCEF genomic and transcriptomic microarray study of the response to radiotherapy or to alkylating first-line chemotherapy in glioblastoma patients. *Mol Cancer, 9*, 234. doi:10.1186/1476-4598-9-234

Dürst, M., Gissmann, L., Ikenberg, H., & zur Hausen, H. (1983). A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. *Proc Natl Acad Sci U S A, 80*(12), 3812-3815. doi:10.1073/pnas.80.12.3812

Eisbruch, A., Harris, J., Garden, A. S., Chao, C. K., Straube, W., Harari, P. M., . . . Ang, K. K. (2010). Multi-institutional trial of accelerated hypofractionated intensity-modulated radiation therapy for early-stage oropharyngeal cancer (RTOG 00-22). *International Journal of Radiation Oncology* Biology* Physics, 76*(5), 1333-1338.

Eisbruch, A., Ten Haken, R. K., Kim, H. M., Marsh, L. H., & Ship, J. A. (1999). Dose, volume, and function relationships in parotid salivary glands following conformal and intensity-modulated irradiation of head and neck cancer. *Int J Radiat Oncol Biol Phys, 45*(3), 577-587. doi:10.1016/s0360-3016(99)00247-3

Epstein, J. B., Wong, F. L., Dickens, A., Szasz, I., & Lepawsky, M. (1992). Bone and gallium scans in postradiotherapy osteonecrosis of the jaw. *Head Neck, 14*(4), 288-292. doi:10.1002/hed.2880140406

Ernster, J. A., Sciotto, C. G., O'Brien, M. M., Finch, J. L., Robinson, L. J., Willson, T., & Mathews, M. (2007). Rising incidence of oropharyngeal cancer and the role of oncogenic human papilloma virus. *Laryngoscope, 117*(12), 2115-2128. doi:10.1097/MLG.0b013e31813e5fbb

Fajardo, L., Berthrong, M., & Anderson, R. (2001). *Radiation pathology*: Oxford University Press.

Fakhry, C., Westra, W. H., Li, S., Cmelak, A., Ridge, J. A., Pinto, H., . . . Gillison, M. L. (2008). Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *J Natl Cancer Inst, 100*(4), 261-269. doi:10.1093/jnci/djn011

Fast, D. K., Felix, R., Dowse, C., Neuman, W., & Fleisch, H. (1978). The effects of diphosphonates on the growth and glycolysis of connective-tissue cells in culture. *Biochemical Journal, 172*(1), 97-107.

Fenner, M., Park, J., Schulz, N., Amann, K., Grabenbauer, G. G., Fahrig, A., . . . Nkenke, E. (2010). Validation of histologic changes induced by external irradiation in mandibular bone. An experimental animal model. *Journal of Cranio-Maxillofacial Surgery, 38*(1), 47-53.

Ferlay, J., Shin, H. R., Bray, F., Forman, D., Mathers, C., & Parkin, D. M. (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *International journal of cancer, 127*(12), 2893-2917. doi:10.1002/ijc.25516

Franceschi, S., Bidoli, E., Herrero, R., & Muñoz, N. (2000). Comparison of cancers of the oral cavity and pharynx worldwide: etiological clues. *Oral Oncol, 36*(1), 106-115. doi:10.1016/s1368-8375(99)00070-6

Fromigue, O., & Body, J.-J. (2002). Bisphosphonates influence the proliferation and the maturation of normal human osteoblasts. *Journal of endocrinological investigation, 25*(6), 539-546.

Frydrych, A., & Slack-Smith, L. (2011). Dental attendance of oral and oropharyngeal cancer patients in a public hospital in Western Australia. *Australian dental journal, 56*(3), 278-283.

Furness, S., Glenny, A. M., Worthington, H. V., Pavitt, S., Oliver, R., Clarkson, J. E., . . . Conway, D. I. (2010). Interventions for the treatment of oral cavity and oropharyngeal

cancer: chemotherapy. *Cochrane Database Syst Rev*(9), Cd006386. doi:10.1002/14651858.CD006386.pub2
Gadiwalla, Y., & Patel, V. (2018). Osteonecrosis of the jaw unrelated to medication or radiotherapy. *Oral surgery, oral medicine, oral pathology and oral radiology, 125*(5), 446-453.
Gaggenheimer, J., & Hoffman, R. D. (1994). The importance of screening edentulous patients for oral cancer. *Journal of Prosthetic Dentistry, 72*(2), 141-143.
Galon, J., Costes, A., Sanchez-Cabo, F., Kirilovsky, A., Mlecnik, B., Lagorce-Pagès, C., . . . Pagès, F. (2006). Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science, 313*(5795), 1960-1964. doi:10.1126/science.1129139
Galon, J., Mlecnik, B., Bindea, G., Angell, H. K., Berger, A., Lagorce, C., . . . Pagès, F. (2014). Towards the introduction of the 'Immunoscore' in the classification of malignant tumours. *J Pathol, 232*(2), 199-209. doi:10.1002/path.4287
Garg, H., Grewal, M. S., Rawat, S., Suhag, A., Sood, P. B., Grewal, S., & Ahlawat, P. (2015). Dental Pulp Status of Posterior Teeth in Patients with Oral and Oropharyngeal Cancer Treated with Concurrent Chemoradiotherapy. *J Endod, 41*(11), 1830-1833. doi:10.1016/j.joen.2015.08.006
Gevorgyan, A., Wong, K., Poon, I., Blanas, N., Enepekides, D. J., & Higgins, K. M. (2013). Osteoradionecrosis of the mandible: a case series at a single institution. *Journal of Otolaryngology-Head & Neck Surgery, 42*(1), 46.
Gillison, M. L., Alemany, L., Snijders, P. J., Chaturvedi, A., Steinberg, B. M., Schwartz, S., & Castellsagué, X. (2012). Human papillomavirus and diseases of the upper airway: head and neck cancer and respiratory papillomatosis. *Vaccine, 30 Suppl 5*, F34-54. doi:10.1016/j.vaccine.2012.05.070
Gillison, M. L., Chaturvedi, A. K., Anderson, W. F., & Fakhry, C. (2015). Epidemiology of Human Papillomavirus-Positive Head and Neck Squamous Cell Carcinoma. *J Clin Oncol, 33*(29), 3235-3242. doi:10.1200/jco.2015.61.6995
Gillison, M. L., D'Souza, G., Westra, W., Sugar, E., Xiao, W., Begum, S., & Viscidi, R. (2008). Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. *J Natl Cancer Inst, 100*(6), 407-420. doi:10.1093/jnci/djn025
Gillison, M. L., Zhang, Q., Jordan, R., Xiao, W., Westra, W. H., Trotti, A., . . . Ang, K. K. (2012). Tobacco smoking and increased risk of death and progression for patients with p16-positive and p16-negative oropharyngeal cancer. *J Clin Oncol, 30*(17), 2102-2111. doi:10.1200/jco.2011.38.4099
Gladkova, N., Maslennikova, A., Balalaeva, I., Feldchtein, F., Kiseleva, E., Karabut, M., & Iksanov, R. (2008). Application of optical coherence tomography in the diagnosis of mucositis in patients with head and neck cancer during a course of radio(chemo)therapy. *Medical Laser Application, 23*(4), 186-195. doi:https://doi.org/10.1016/j.mla.2008.07.008
Glanzmann, C., & Grätz, K. W. (1995). Radionecrosis of the mandibula: a retrospective analysis of the incidence and risk factors. *Radiother Oncol, 36*(2), 94-100. doi:10.1016/0167-8140(95)01583-3
Gomez, D. R., Estilo, C. L., Wolden, S. L., Zelefsky, M. J., Kraus, D. H., Wong, R. J., . . . Lee, N. Y. (2011). Correlation of osteoradionecrosis and dental events with dosimetric parameters in intensity-modulated radiation therapy for head-and-neck cancer. *International Journal of Radiation Oncology* Biology* Physics, 81*(4), e207-e213.

Gooden, M. J., de Bock, G. H., Leffers, N., Daemen, T., & Nijman, H. W. (2011). The prognostic influence of tumour-infiltrating lymphocytes in cancer: a systematic review with meta-analysis. *Br J Cancer, 105*(1), 93-103. doi:10.1038/bjc.2011.189

Grabenbauer, G. G., Lahmer, G., Distel, L., & Niedobitek, G. (2006). Tumor-infiltrating cytotoxic T cells but not regulatory T cells predict outcome in anal squamous cell carcinoma. *Clin Cancer Res, 12*(11 Pt 1), 3355-3360. doi:10.1158/1078-0432.Ccr-05-2434

Graff, P., Lapeyre, M., Desandes, E., Ortholan, C., Bensadoun, R. J., Alfonsi, M., . . . Peiffert, D. (2007). Impact of intensity-modulated radiotherapy on health-related quality of life for head and neck cancer patients: matched-pair comparison with conventional radiotherapy. *Int J Radiat Oncol Biol Phys, 67*(5), 1309-1317. doi:10.1016/j.ijrobp.2006.11.012

Granata, R., Miceli, R., Orlandi, E., Perrone, F., Cortelazzi, B., Franceschini, M., . . . Licitra, L. (2012). Tumor stage, human papillomavirus and smoking status affect the survival of patients with oropharyngeal cancer: an Italian validation study. *Ann Oncol, 23*(7), 1832-1837. doi:10.1093/annonc/mdr544

Grant, B. P., & Fletcher, G. H. (1966). Analysis of complications following megavoltage therapy for squamous cell carcinomas of the tonsillar area. *American Journal of Roentgenology, 96*(1), 28-36.

Greene, F. L., Balch, C. M., Fleming, I. D., Fritz, A., Haller, D. G., Morrow, M., & Page, D. L. (2002). *AJCC cancer staging handbook: TNM classification of malignant tumors*: Springer Science & Business Media.

Grønhøj, C., Jensen, D., Dehlendorff, C., Nørregaard, C., Andersen, E., Specht, L., . . . Von Buchwald, C. (2018). Impact of Time to Treatment Initiation in Patients with Human Papillomavirus-positive and-negative Oropharyngeal Squamous Cell Carcinoma. *Clinical Oncology, 30*(6), 375-381.

Guo, T., Goldenberg, D., & Fakhry, C. (2017). AHNS series: Do you know your guidelines? Management of head and neck cancer in the era of human papillomavirus: Educating our patients on human papillomavirus. *Head Neck, 39*(5), 833-839. doi:10.1002/hed.24693

Gupta, T., Agarwal, J., Jain, S., Phurailatpam, R., Kannan, S., Ghosh-Laskar, S., . . . D'Cruz, A. (2012). Three-dimensional conformal radiotherapy (3D-CRT) versus intensity modulated radiation therapy (IMRT) in squamous cell carcinoma of the head and neck: a randomized controlled trial. *Radiother Oncol, 104*(3), 343-348. doi:10.1016/j.radonc.2012.07.001

Gupta, T., Jain, S., Agarwal, J. P., Ghosh-Laskar, S., Phurailatpam, R., Pai-Shetty, R., & Dinshaw, K. A. (2011). Prospective assessment of patterns of failure after high-precision definitive (chemo)radiation in head-and-neck squamous cell carcinoma. *Int J Radiat Oncol Biol Phys, 80*(2), 522-531. doi:10.1016/j.ijrobp.2010.01.054

Gupta, T., Kannan, S., Ghosh-Laskar, S., & Agarwal, J. P. (2018). Systematic review and meta-analyses of intensity-modulated radiation therapy versus conventional two-dimensional and/or or three-dimensional radiotherapy in curative-intent management of head and neck squamous cell carcinoma. *PLoS One, 13*(7), e0200137. doi:10.1371/journal.pone.0200137

Hammarstedt, L., Lindquist, D., Dahlstrand, H., Romanitan, M., Dahlgren, L. O., Joneberg, J., . . . Munck-Wikland, E. (2006). Human papillomavirus as a risk factor for the increase in incidence of tonsillar cancer. *International journal of cancer, 119*(11), 2620-2623. doi:10.1002/ijc.22177

Hansen, H. J., Maritim, B., Bohle, G. C., 3rd, Lee, N. Y., Huryn, J. M., & Estilo, C. L. (2012). Dosimetric distribution to the tooth-bearing regions of the mandible following

intensity-modulated radiation therapy for base of tongue cancer. *Oral Surg Oral Med Oral Pathol Oral Radiol, 114*(2), e50-54. doi:10.1016/j.oooo.2012.01.024
Haria, S., Patel, V., Sproat, C., Kwok, J., Ormondroyd, L., McGurk, M., . . . Cascarini, L. (2016). Is Osteoradionecrosis Evolving with Improved Radiotherapy Delivery System? *Journal of oral and maxillofacial surgery, 74*(9), e37.
Harris, M. (1992). The conservative management of osteoradionecrosis of the mandible with ultrasound therapy. *Br J Oral Maxillofac Surg, 30*(5), 313-318. doi:10.1016/0266-4356(92)90181-h
Hashibe, M., Brennan, P., Benhamou, S., Castellsague, X., Chen, C., Curado, M. P., . . . Boffetta, P. (2007). Alcohol drinking in never users of tobacco, cigarette smoking in never drinkers, and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. *J Natl Cancer Inst, 99*(10), 777-789. doi:10.1093/jnci/djk179
Hashibe, M., Brennan, P., Chuang, S. C., Boccia, S., Castellsague, X., Chen, C., . . . Boffetta, P. (2009). Interaction between tobacco and alcohol use and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. *Cancer Epidemiol Biomarkers Prev, 18*(2), 541-550. doi:10.1158/1055-9965.Epi-08-0347
Helmers, R., Straat, N. F., Navran, A., Nai Chung Tong, T. A. P., Teguh, D. N., van Hulst, R. A., . . . Milstein, D. M. J. (2018). Patient-Side Appraisal of Late Radiation-Induced Oral Microvascular Changes. *Int J Radiat Oncol Biol Phys, 102*(4), 1299-1307. doi:10.1016/j.ijrobp.2018.01.051
Hill, R. P., Rodemann, H. P., Hendry, J. H., Roberts, S. A., & Anscher, M. S. (2001). Normal tissue radiobiology: from the laboratory to the clinic. *Int J Radiat Oncol Biol Phys, 49*(2), 353-365. doi:10.1016/s0360-3016(00)01484-x
Honess, D. J., Dennis, I. F., & Bleehen, N. M. (1993). Pentoxifylline: its pharmacokinetics and ability to improve tumour perfusion and radiosensitivity in mice. *Radiotherapy and Oncology, 28*(3), 208-218.
Hong, Martin, A., Chatfield, M., Jones, D., Zhang, M., Armstrong, B., . . . Clark, J. (2013). Human papillomavirus, smoking status and outcomes in tonsillar squamous cell carcinoma. *International journal of cancer, 132*(12), 2748-2754.
Hong, A. M., Grulich, A. E., Jones, D., Lee, C. S., Garland, S. M., Dobbins, T. A., . . . Rose, B. R. (2010). Squamous cell carcinoma of the oropharynx in Australian males induced by human papillomavirus vaccine targets. *Vaccine, 28*(19), 3269-3272. doi:10.1016/j.vaccine.2010.02.098
Huang, K., Xia, P., Chuang, C., Weinberg, V., Glastonbury, C. M., Eisele, D. W., . . . Quivey, J. M. (2008). Intensity-modulated chemoradiation for treatment of stage III and IV oropharyngeal carcinoma: the University of California–San Francisco experience. *Cancer: Interdisciplinary International Journal of the American Cancer Society, 113*(3), 497-507.
Hutchinson, I. (1996). Complications of radiotherapy in the head and neck: an orofacial surgeon's view. *Current radiation oncology. Arnold, London*, 144-177.
IMRTCWG. (2001). Intensity-modulated radiotherapy: current status and issues of interest. *Int J Radiat Oncol Biol Phys, 51*(4), 880-914. doi:10.1016/s0360-3016(01)01749-7
ISRCTN. (2018). De-ESCALaTE HPV: Determination of Epidermal growth factor receptor-inhibitor (cetuximab) versus Standard Chemotherapy (cisplatin) early And Late Toxicity Events in Human Papillomavirus-positive oropharyngeal squamous cell carcinoma. Retrieved from http://www.isrctn.com/ISRCTN33522080
ISRCTN. (2019). A clinic feasibility study to assess whether the use of two combined medicines (pentoxifylline and tocopherol) can prevent radiotherapy-related changes

of the mouth and face compared to the current standard of care in the head and neck cancer population. Retrieved from http://www.isrctn.com/ISRCTN74484952?q=&filters=conditionCategory:Cancer,publicationStatus:withoutProtocol&sort=&offset=7&totalResults=1980&page=1&pageSize=10&searchType=basic-search

ISRCTN. (2020). Comparing alternative regimens for escalating treatment of intermediate and high-risk oropharyngeal cancer. Retrieved from http://www.isrctn.com/ISRCTN41478539

Jellema, A. P., Slotman, B. J., Doornaert, P., Leemans, C. R., & Langendijk, J. A. (2007). Impact of radiation-induced xerostomia on quality of life after primary radiotherapy among patients with head and neck cancer. *Int J Radiat Oncol Biol Phys, 69*(3), 751-760. doi:10.1016/j.ijrobp.2007.04.021

Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E., & Forman, D. (2011). Global cancer statistics. *CA: a cancer journal for clinicians, 61*(2), 69-90.

Jemal, A., Siegel, R., Ward, E., Hao, Y., Xu, J., Murray, T., & Thun, M. J. (2008). Cancer statistics, 2008. *CA Cancer J Clin, 58*(2), 71-96. doi:10.3322/ca.2007.0010

Jham, B. C., Reis, P. M., Miranda, E. L., Lopes, R. C., Carvalho, A. L., Scheper, M. A., & Freire, A. R. (2008). Oral health status of 207 head and neck cancer patients before, during and after radiotherapy. *Clin Oral Investig, 12*(1), 19-24. doi:10.1007/s00784-007-0149-5

JHNT-MRI-DC. (2016). Dynamic contrast-enhanced MRI detects acute radiotherapy-induced alterations in mandibular microvasculature: prospective assessment of imaging biomarkers of normal tissue injury. *Sci Rep, 6*, 29864. doi:10.1038/srep29864

Kasibhatla, M., Kirkpatrick, J. P., & Brizel, D. M. (2007). How much radiation is the chemotherapy worth in advanced head and neck cancer? *International Journal of Radiation Oncology* Biology* Physics, 68*(5), 1491-1495.

Keck, M. K., Zuo, Z., Khattri, A., Stricker, T. P., Brown, C. D., Imanguli, M., . . . Seiwert, T. Y. (2015). Integrative analysis of head and neck cancer identifies two biologically distinct HPV and three non-HPV subtypes. *Clin Cancer Res, 21*(4), 870-881. doi:10.1158/1078-0432.Ccr-14-2481

Kojima, Y., Yanamoto, S., Umeda, M., Kawashita, Y., Saito, I., Hasegawa, T., . . . Yamada, S.-i. (2017). Relationship between dental status and development of osteoradionecrosis of the jaw: a multicenter retrospective study. *Oral surgery, oral medicine, oral pathology and oral radiology, 124*(2), 139-145.

Kreimer, A. R., Bhatia, R. K., Messeguer, A. L., Gonzalez, P., Herrero, R., & Giuliano, A. R. (2010). Oral human papillomavirus in healthy individuals: a systematic review of the literature. *Sex Transm Dis, 37*(6), 386-391. doi:10.1097/OLQ.0b013e3181c94a3b

Kreimer, A. R., Clifford, G. M., Boyle, P., & Franceschi, S. (2005). Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer Epidemiol Biomarkers Prev, 14*(2), 467-475. doi:10.1158/1055-9965.Epi-04-0551

Kuhnt, T., Stang, A., Wienke, A., Vordermark, D., Schweyen, R., & Hey, J. (2016). Potential risk factors for jaw osteoradionecrosis after radiotherapy for head and neck cancer. *Radiat Oncol, 11*, 101. doi:10.1186/s13014-016-0679-6

Langendijk, J. A., Doornaert, P., Verdonck-de Leeuw, I. M., Leemans, C. R., Aaronson, N. K., & Slotman, B. J. (2008). Impact of late treatment-related toxicity on quality of life among patients with head and neck cancer treated with radiotherapy. *J Clin Oncol, 26*(22), 3770-3776. doi:10.1200/jco.2007.14.6647

Lee, J., Koom, W. S., Lee, C. G., Kim, Y. B., Yoo, S. W., Keum, K. C., . . . Cha, I. H. (2009). Risk factors and dose–effect relationship for mandibular osteoradionecrosis in oral

and oropharyngeal cancer patients. *International Journal of Radiation Oncology* Biology* Physics, 75*(4), 1084-1091.
Lee, N., Xia, P., Quivey, J. M., Sultanem, K., Poon, I., Akazawa, C., . . . Fu, K. K. (2002). Intensity-modulated radiotherapy in the treatment of nasopharyngeal carcinoma: an update of the UCSF experience. *Int J Radiat Oncol Biol Phys, 53*(1), 12-22. doi:10.1016/s0360-3016(02)02724-4
Li, W., Thompson, C. H., O'Brien, C. J., McNeil, E. B., Scolyer, R. A., Cossart, Y. E., . . . Rose, B. R. (2003). Human papillomavirus positivity predicts favourable outcome for squamous carcinoma of the tonsil. *International journal of cancer, 106*(4), 553-558. doi:10.1002/ijc.11261
Lindquist, D., Romanitan, M., Hammarstedt, L., Näsman, A., Dahlstrand, H., Lindholm, J., . . . Dalianis, T. (2007). Human papillomavirus is a favourable prognostic factor in tonsillar cancer and its oncogenic role is supported by the expression of E6 and E7. *Mol Oncol, 1*(3), 350-355. doi:10.1016/j.molonc.2007.08.005
Liu, Y., Lim, J., & Teoh, S. H. (2013). Review: development of clinically relevant scaffolds for vascularised bone tissue engineering. *Biotechnol Adv, 31*(5), 688-705. doi:10.1016/j.biotechadv.2012.10.003
Lockhart, P. B., & Clark, J. (1994). Pretherapy dental status of patients with malignant conditions of the head and neck. *Oral surgery, oral medicine, oral pathology, 77*(3), 236-241.
Louie, K. S., Mehanna, H., & Sasieni, P. (2015). Trends in head and neck cancers in England from 1995 to 2011 and projections up to 2025. *Oral Oncol, 51*(4), 341-348. doi:10.1016/j.oraloncology.2015.01.002
MacComb, W. (1962). Necrosis in treatment of intraoral cancer by radiation therapy. *Am. J. Roentgenol., Radium Therapy Nuclear Med., 87*.
Maesschalck, T., Dulguerov, N., Caparrotti, F., Scolozzi, P., Picardi, C., Mach, N., . . . Dulguerov, P. (2016). Comparison of the incidence of osteoradionecrosis with conventional radiotherapy and intensity-modulated radiotherapy. *Head Neck, 38*(11), 1695-1702. doi:10.1002/hed.24505
Maier, H., Zöller, J., Herrmann, A., Kreiss, M., & Heller, W.-D. (1993). Dental status and oral hygiene in patients with head and neck cancer. *Otolaryngology—Head and Neck Surgery, 108*(6), 655-661.
Mainous, E. G., Boyne, P. J., & Hart, G. B. (1973). Elimination of sequestrum and healing of osteoradionecrosis of the mandible after hyperbaric oxygen therapy: report of case. *J Oral Surg, 31*(5), 336-339.
Manicone, P., Tarli, C., Mirijello, A., Raffaelli, L., Vassallo, G., Antonelli, M., . . . Lavorgna, L. (2017). Dental health in patients affected by alcohol use disorders: a cross-sectional study. *Eur Rev Med Pharmacol Sci, 21*(22), 5021-5027.
Markiewicz, D. A., Schultz, D. J., Haas, J. A., Harris, E. E., Fox, K. R., Glick, J. H., & Solin, L. J. (1996). The effects of sequence and type of chemotherapy and radiation therapy on cosmesis and complications after breast conservation therapy. *Int J Radiat Oncol Biol Phys, 35*(4), 661-668. doi:10.1016/0360-3016(96)00171-x
Martín-Hernán, F., Sánchez-Hernández, J.-G., Cano, J., Campo, J., & del Romero, J. (2013). Oral cancer, HPV infection and evidence of sexual transmission. *Medicina oral, patologia oral y cirugia bucal, 18*(3), e439-e444. doi:10.4317/medoral.18419
Martos-Fernández, M., Saez-Barba, M., López-López, J., Estrugo-Devesa, A., Balibrea-del-Castillo, J. M., & Bescós-Atín, C. (2018). Pentoxifylline, tocopherol, and clodronate for the treatment of mandibular osteoradionecrosis: a systematic review. *Oral surgery, oral medicine, oral pathology and oral radiology, 125*(5), 431-439.

Marur, S., D'Souza, G., Westra, W. H., & Forastiere, A. A. (2010). HPV-associated head and neck cancer: a virus-related cancer epidemic. *Lancet Oncol, 11*(8), 781-789. doi:10.1016/s1470-2045(10)70017-6

Marur, S., & Forastiere, A. A. (2008). Head and neck cancer: changing epidemiology, diagnosis, and treatment. *Mayo Clin Proc, 83*(4), 489-501. doi:10.4065/83.4.489

Marx, R., & Tursun, R. (2012). Suppurative osteomyelitis, bisphosphonate induced osteonecrosis, osteoradionecrosis: a blinded histopathologic comparison and its implications for the mechanism of each disease. *International journal of oral and maxillofacial surgery, 41*(3), 283-289.

Marx, R. E. (1983). A new concept in the treatment of osteoradionecrosis. *J Oral Maxillofac Surg, 41*(6), 351-357. doi:10.1016/s0278-2391(83)80005-6

Maslennikova, A., Sirotkina, M., Moiseev, A., Finagina, E., Ksenofontov, S., Gelikonov, G., . . . Zagaynova, E. (2017). In-vivo longitudinal imaging of microvascular changes in irradiated oral mucosa of radiotherapy cancer patients using optical coherence tomography. *Scientific reports, 7*(1), 1-10.

Masterson, L., Moualed, D., Liu, Z. W., Howard, J. E., Dwivedi, R. C., Tysome, J. R., . . . Goon, P. K. (2014). De-escalation treatment protocols for human papillomavirus-associated oropharyngeal squamous cell carcinoma: a systematic review and meta-analysis of current clinical trials. *Eur J Cancer, 50*(15), 2636-2648. doi:10.1016/j.ejca.2014.07.001

Matlung, S. E., Wilhelmina van Kempen, P. M., Bovenschen, N., van Baarle, D., & Willems, S. M. (2016). Differences in T-cell infiltrates and survival between HPV+ and HPV- oropharyngeal squamous cell carcinoma. *Future science OA, 2*(1), FSO88-FSO88. doi:10.4155/fso.15.88

McArdle, L. W., & Renton, T. (2012). The effects of NICE guidelines on the management of third molar teeth. *Br Dent J, 213*(5), E8. doi:10.1038/sj.bdj.2012.780

McGregor, A., & MacDonald, D. (1995). Post-irradiation changes in the blood vessels of the adult human mandible. *British Journal of Oral and Maxillofacial Surgery, 33*(1), 15-18.

MDAndersonHNCSWG. (2017). Dose-volume correlates of mandibular osteoradionecrosis in Oropharynx cancer patients receiving intensity-modulated radiotherapy: Results from a case-matched comparison. *Radiother Oncol, 124*(2), 232-239. doi:10.1016/j.radonc.2017.06.026

Mehanna, Franklin, N., Compton, N., Robinson, M., Powell, N., Biswas–Baldwin, N., . . . Al-Booz, H. (2016). Geographic variation in human papillomavirus–related oropharyngeal cancer: Data from 4 multinational randomized trials. *Head & neck, 38*(S1), E1863-E1869.

Mehanna, H., Beech, T., Nicholson, T., El-Hariry, I., McConkey, C., Paleri, V., & Roberts, S. (2013). Prevalence of human papillomavirus in oropharyngeal and nonoropharyngeal head and neck cancer--systematic review and meta-analysis of trends by time and region. *Head Neck, 35*(5), 747-755. doi:10.1002/hed.22015

Mehanna., Evans, M., Beasley, M., Chatterjee, S., Dilkes, M., Homer, J., . . . Sloan, P. (2016). Oropharyngeal cancer: United Kingdom National Multidisciplinary Guidelines. *The Journal of laryngology and otology, 130*(S2), S90-S96. doi:10.1017/S0022215116000505

Meyer, I. (1970). Infectious diseases of the jaws. *Journal of oral surgery (American Dental Association: 1965), 28*(1), 17-26.

Michaud, D. S., Langevin, S. M., Eliot, M., Nelson, H. H., Pawlita, M., McClean, M. D., & Kelsey, K. T. (2014). High-risk HPV types and head and neck cancer. *International journal of cancer, 135*(7), 1653-1661. doi:10.1002/ijc.28811

Mirghani, H., & Blanchard, P. (2018). Treatment de-escalation for HPV-driven oropharyngeal cancer: Where do we stand? *Clin Transl Radiat Oncol, 8*, 4-11. doi:10.1016/j.ctro.2017.10.005

Mlecnik, B., Tosolini, M., Kirilovsky, A., Berger, A., Bindea, G., Meatchi, T., . . . Galon, J. (2011). Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. *J Clin Oncol, 29*(6), 610-618. doi:10.1200/jco.2010.30.5425

Montejo, M. E., Shrieve, D. C., Bentz, B. G., Hunt, J. P., Buchman, L. O., Agarwal, N., & Hitchcock, Y. J. (2011). IMRT with simultaneous integrated boost and concurrent chemotherapy for locoregionally advanced squamous cell carcinoma of the head and neck. *International Journal of Radiation Oncology* Biology* Physics, 81*(5), e845-e852.

Moon, D. H., Moon, S. H., Wang, K., Weissler, M. C., Hackman, T. G., Zanation, A. M., . . . Marks, L. B. (2017). Incidence of, and risk factors for, mandibular osteoradionecrosis in patients with oral cavity and oropharynx cancers. *Oral oncology, 72*, 98-103.

Moraes, R. C., Dias, F. L., Figueredo, C. M., & Fischer, R. G. (2016). Association between Chronic Periodontitis and Oral/Oropharyngeal Cancer. *Braz Dent J, 27*(3), 261-266. doi:10.1590/0103-6440201600754

Mork, J., Lie, A. K., Glattre, E., Hallmans, G., Jellum, E., Koskela, P., . . . Dillner, J. (2001). Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. *N Engl J Med, 344*(15), 1125-1131. doi:10.1056/nejm200104123441503

Mork, J., Møller, B., Dahl, T., & Bray, F. (2010). Time trends in pharyngeal cancer incidence in Norway 1981-2005: a subsite analysis based on a reabstraction and recoding of registered cases. *Cancer Causes Control, 21*(9), 1397-1405. doi:10.1007/s10552-010-9567-9

Mork, J., Møller, B., Dahl, T., & Bray, F. (2010). Time trends in pharyngeal cancer incidence in Norway 1981–2005: a subsite analysis based on a reabstraction and recoding of registered cases. *Cancer Causes & Control, 21*(9), 1397-1405.

Morrish Jr, R. B., Chan, E., Silverman Jr, S., Meyer, J., Fu, K. K., & Greenspan, D. (1981). Osteonecrosis in patients irradiated for head and neck carcinoma. *Cancer, 47*(8), 1980-1983.

Muraki, Y., Akashi, M., Ejima, Y., Hasegawa, T., Miyawaki, D., Shinomiya, H., . . . Nibu, K.-i. (2019). Dental intervention against osteoradionecrosis of the jaws in irradiated patients with head and neck malignancy: a single-arm prospective study. *Oral and maxillofacial surgery, 23*(3), 297-305.

Murdoch-Kinch, C. A., Kim, H. M., Vineberg, K. A., Ship, J. A., & Eisbruch, A. (2008). Dose-effect relationships for the submandibular salivary glands and implications for their sparing by intensity modulated radiotherapy. *Int J Radiat Oncol Biol Phys, 72*(2), 373-382. doi:10.1016/j.ijrobp.2007.12.033

Murphy, E. A., Shields, D. J., Stoletov, K., Dneprovskaia, E., McElroy, M., Greenberg, J. I., . . . Majeti, B. K. (2010). Disruption of angiogenesis and tumor growth with an orally active drug that stabilizes the inactive state of PDGFRβ/B-RAF. *Proceedings of the National Academy of Sciences, 107*(9), 4299-4304.

Murray, C. G., Herson, J., Daly, T. E., & Zimmerman, S. (1980). Radiation necrosis of the mandible: a 10 year study. Part I. Factors influencing the onset of necrosis. *International Journal of Radiation Oncology* Biology* Physics, 6*(5), 543-548.

Nabil, S., & Samman, N. (2011). Incidence and prevention of osteoradionecrosis after dental extraction in irradiated patients: a systematic review. *International journal of oral and maxillofacial surgery, 40*(3), 229-243.

Nabil, S., & Samman, N. (2012). Risk factors for osteoradionecrosis after head and neck radiation: A systematic review. *Oral surgery, oral medicine, oral pathology and oral radiology, 113*(1), 54-69.

Narayan, S., Lehmann, J., Coleman, M. A., Vaughan, A., Yang, C. C., Enepekides, D., . . . Vijayakumar, S. (2008). Prospective evaluation to establish a dose response for clinical oral mucositis in patients undergoing head-and-neck conformal radiotherapy. *Int J Radiat Oncol Biol Phys, 72*(3), 756-762. doi:10.1016/j.ijrobp.2008.01.060

Näsman, A., Attner, P., Hammarstedt, L., Du, J., Eriksson, M., Giraud, G., . . . Lindquist, D. (2009). Incidence of human papillomavirus (HPV) positive tonsillar carcinoma in Stockholm, Sweden: an epidemic of viral-induced carcinoma? *International journal of cancer, 125*(2), 362-366.

Ndiaye, Mena, M., Alemany, L., Arbyn, M., Castellsagué, X., Laporte, L., . . . Trottier, H. (2014). HPV DNA, E6/E7 mRNA, and p16INK4a detection in head and neck cancers: a systematic review and meta-analysis. *The Lancet Oncology, 15*(12), 1319-1331.

Ndiaye, C., Mena, M., Alemany, L., Arbyn, M., Castellsague, X., Laporte, L., . . . Trottier, H. (2014). HPV DNA, E6/E7 mRNA, and p16INK4a detection in head and neck cancers: a systematic review and meta-analysis. *Lancet Oncol, 15*(12), 1319-1331. doi:10.1016/s1470-2045(14)70471-1

Nedergaard, B. S., Ladekarl, M., Thomsen, H. F., Nyengaard, J. R., & Nielsen, K. (2007). Low density of CD3+, CD4+ and CD8+ cells is associated with increased risk of relapse in squamous cell cervical cancer. *British journal of cancer, 97*(8), 1135-1138. doi:10.1038/sj.bjc.6604001

Nguyen, N. P., Vock, J., Chi, A., Ewell, L., Vos, P., Mills, M., . . . Betz, M. (2012). Effectiveness of intensity-modulated and image-guided radiotherapy to spare the mandible from excessive radiation. *Oral oncology, 48*(7), 653-657.

NHS. (2015). Statistics on Smoking, England - 2015. Retrieved from https://digital.nhs.uk/data-and-information/publications/statistical/statistics-on-smoking/statistics-on-smoking-england-2015

NICE. (2000). Guidance on the Extraction of Wisdom Teeth. Retrieved from https://www.nice.org.uk/guidance/ta1/resources/guidance-on-the-extraction-of-wisdom-teeth-pdf-63732983749

NICE. (2004). Improving Outcomes in Head and Neck Cancers. Retrieved from https://www.nice.org.uk/guidance/csg6/resources/improving-outcomes-in-head-and-neck-cancers-update.pdf-773377597

NICE. (2018). Cancer of the upper aerodigestive tract: assessment and management in people aged 16 and over. Retrieved from https://www.nice.org.uk/guidance/ng36/chapter/Recommendations#hpvrelated-disease.

Niewald, M., Fleckenstein, J., Mang, K., Holtmann, H., Spitzer, W. J., & Rübe, C. (2013). Dental status, dental rehabilitation procedures, demographic and oncological data as potential risk factors for infected osteoradionecrosis of the lower jaw after radiotherapy for oral neoplasms: a retrospective evaluation. *Radiat Oncol, 8*, 227. doi:10.1186/1748-717x-8-227

Nordfors, C., Grün, N., Tertipis, N., Ährlund-Richter, A., Haeggblom, L., Sivars, L., . . . Dalianis, T. (2013). CD8+ and CD4+ tumour infiltrating lymphocytes in relation to human papillomavirus status and clinical outcome in tonsillar and base of tongue squamous cell carcinoma. *Eur J Cancer, 49*(11), 2522-2530. doi:10.1016/j.ejca.2013.03.019

Nutting, C. M., Morden, J. P., Harrington, K. J., Urbano, T. G., Bhide, S. A., Clark, C., . . . Hall, E. (2011). Parotid-sparing intensity modulated versus conventional radiotherapy

in head and neck cancer (PARSPORT): a phase 3 multicentre randomised controlled trial. *Lancet Oncol, 12*(2), 127-136. doi:10.1016/s1470-2045(10)70290-4
O'Sullivan, B., Huang, S. H., Perez-Ordonez, B., Massey, C., Siu, L. L., Weinreb, I., . . . Xu, W. (2012). Outcomes of HPV-related oropharyngeal cancer patients treated by radiotherapy alone using altered fractionation. *Radiother Oncol, 103*(1), 49-56. doi:10.1016/j.radonc.2012.02.009
Oguejiofor, K., Hall, J., Slater, C., Betts, G., Hall, G., Slevin, N., . . . West, C. M. (2015). Stromal infiltration of CD8 T cells is associated with improved clinical outcome in HPV-positive oropharyngeal squamous carcinoma. *Br J Cancer, 113*(6), 886-893. doi:10.1038/bjc.2015.277
ONS. (2011). General Lifestyle Survey, 2011. Retrieved from https://www.ons.gov.uk/peoplepopulationandcommunity/personalandhouseholdfinances/incomeandwealth/compendium/generallifestylesurvey/2013-03-07/generallifestylesurvey2011referencetables
ONS. (2019). Cancer registration statistics, England Statistical bulletins. Retrieved from https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/bulletins/cancerregistrationstatisticsengland/previousReleases
Owosho, A., Tsai, C. J., Lee, R. S., Freymiller, H., Kadempour, A., Varthis, S., . . . Randazzo, J. (2017). The prevalence and risk factors associated with osteoradionecrosis of the jaw in oral and oropharyngeal cancer patients treated with intensity-modulated radiation therapy (IMRT): The Memorial Sloan Kettering Cancer Center experience. *Oral oncology, 64*, 44-51.
Owosho, A. A., Yom, S. K., Han, Z., Sine, K., Lee, N. Y., Huryn, J. M., & Estilo, C. L. (2016). Comparison of mean radiation dose and dosimetric distribution to tooth-bearing regions of the mandible associated with proton beam radiation therapy and intensity-modulated radiation therapy for ipsilateral head and neck tumor. *Oral Surg Oral Med Oral Pathol Oral Radiol, 122*(5), 566-571. doi:10.1016/j.oooo.2016.07.003
Packer, L., Weber, S. U., & Rimbach, G. (2001). Molecular aspects of α-tocotrienol antioxidant action and cell signalling. *The Journal of nutrition, 131*(2), 369S-373S.
Panwar, A., Batra, R., Lydiatt, W. M., & Ganti, A. K. (2014). Human papilloma virus positive oropharyngeal squamous cell carcinoma: a growing epidemic. *Cancer Treat Rev, 40*(2), 215-219. doi:10.1016/j.ctrv.2013.09.006
Parahyba, C. J., Ynoe Moraes, F., Ramos, P. A., Haddad, C. M., da Silva, J. L., & Fregnani, E. R. (2016). Radiation dose distribution in the teeth, maxilla, and mandible of patients with oropharyngeal and nasopharyngeal tumors who were treated with intensity-modulated radiotherapy. *Head Neck, 38*(11), 1621-1627. doi:10.1002/hed.24479
Patel, V. (2016). *Re-defining osteoradionecrosis*. Thesis upgrade report. KCL.
Patel, V. (2019). *Osteoradionecrosis in oropharyngeal cancer patients receiving IMRT at GSTT*.
Patel, V., Anton, L., Alim, M., Balasundram, A., Gunaratnam, L., O'neill, M., . . . Vidan, L. H. (2019). A Digitally Generated 3D Dental Model for Assessment of Radiotherapy Doses to the Dentition in Oropharyngeal Cancer Patients for Pre-IMRT Dental Treatment Planning. *Journal of oral and maxillofacial surgery, 77*(9), e32-e33.
Patel, V., Gadiwalla, Y., Sassoon, I., Sproat, C., Kwok, J., & McGurk, M. (2016a). Prophylactic use of pentoxifylline and tocopherol in patients who require dental extractions after radiotherapy for cancer of the head and neck. *Br J Oral Maxillofac Surg, 54*(5), 547-550. doi:10.1016/j.bjoms.2016.02.024

Patel, V., Gadiwalla, Y., Sassoon, I., Sproat, C., Kwok, J., & McGurk, M. (2016b). Use of pentoxifylline and tocopherol in the management of osteoradionecrosis. *British Journal of Oral and Maxillofacial Surgery, 54*(3), 342-345.

Patel, V., & McGurk, M. (2017). Use of pentoxifylline and tocopherol in radiation-induced fibrosis and fibroatrophy. *Br J Oral Maxillofac Surg, 55*(3), 235-241. doi:10.1016/j.bjoms.2016.11.323

Patel, V., & McGurk, M. (2017). Use of pentoxifylline and tocopherol in radiation-induced fibrosis and fibroatrophy. *British Journal of Oral and Maxillofacial Surgery, 55*(3), 235-241.

Patel, V., Ormondroyd, L., Lyons, A., & McGurk, M. (2017). The financial burden for the surgical management of osteoradionecrosis. *Br Dent J, 222*(3), 177-180. doi:10.1038/sj.bdj.2017.121

Patel, V., Patel, D., Browning, T., McGurk, M., Sassoon, I., Gurrero-Urbano, T., & Fenlon, M. (2020). Pre-radiotherapy presenting dental status of the most common head and neck cancer sub-sites in a novel radiation era. . *Faculty Dental Journal, In Press*.

Patel, V., Patel, D., Browning, T., McGurk, M., Sassoon, I., Gurrero-Urbano, T., & Fenlon, M. (2020). Presenting pre-radiotherapy dental status of head and neck cancer patients in the novel radiation era. *British dental journal*(In press).

Patel, V., Young, H., White, T., & Mcgurk, M. (2018). Patient-reported side effects from liquid formulation of pentoxifylline and tocopherol in head and neck radiotherapy patients: an institutional experience and retrospective analysis. *Oral Surgery, 11*(3), 168-174.

Peng, G., Wang, T., Yang, K. Y., Zhang, S., Zhang, T., Li, Q., . . . Wu, G. (2012). A prospective, randomized study comparing outcomes and toxicities of intensity-modulated radiotherapy vs. conventional two-dimensional radiotherapy for the treatment of nasopharyngeal carcinoma. *Radiother Oncol, 104*(3), 286-293. doi:10.1016/j.radonc.2012.08.013

Perry, M. E. (1994). The specialised structure of crypt epithelium in the human palatine tonsil and its functional significance. *J Anat, 185 (Pt 1)*, 111-127.

Pignon, J. P., le Maitre, A., Maillard, E., & Bourhis, J. (2009). Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): an update on 93 randomised trials and 17,346 patients. *Radiother Oncol, 92*(1), 4-14. doi:10.1016/j.radonc.2009.04.014

Poort, Ludlage, J. H. B., Lie, N., Böckmann, R. A., Odekerken, J. C. E., Hoebers, F. J., & Kessler, P. (2017). The histological and histomorphometric changes in the mandible after radiotherapy: An animal model. *J Craniomaxillofac Surg, 45*(5), 716-721. doi:10.1016/j.jcms.2017.02.014

Poort, L. J., Ludlage, J. H., Lie, N., Böckmann, R. A., Odekerken, J. C., Hoebers, F. J., & Kessler, P. A. (2017). The histological and histomorphometric changes in the mandible after radiotherapy: An animal model. *Journal of Cranio-Maxillofacial Surgery, 45*(5), 716-721.

Pytynia, K. B., Dahlstrom, K. R., & Sturgis, E. M. (2014). Epidemiology of HPV-associated oropharyngeal cancer. *Oral Oncol, 50*(5), 380-386. doi:10.1016/j.oraloncology.2013.12.019

Rad, M., Kakoie, S., Niliye Brojeni, F., & Pourdamghan, N. (2010). Effect of Long-term Smoking on Whole-mouth Salivary Flow Rate and Oral Health. *Journal of dental research, dental clinics, dental prospects, 4*(4), 110-114. doi:10.5681/joddd.2010.028

Ragin, C. C., & Taioli, E. (2007). Survival of squamous cell carcinoma of the head and neck in relation to human papillomavirus infection: review and meta-analysis. *International journal of cancer, 121*(8), 1813-1820. doi:10.1002/ijc.22851

Raguse, J. D., Hossamo, J., Tinhofer, I., Hoffmeister, B., Budach, V., Jamil, B., . . . Stromberger, C. (2016). Patient and treatment-related risk factors for osteoradionecrosis of the jaw in patients with head and neck cancer. *Oral Surg Oral Med Oral Pathol Oral Radiol, 121*(3), 215-221.e211. doi:10.1016/j.oooo.2015.10.006

Ramqvist, T., & Dalianis, T. (2010). Oropharyngeal cancer epidemic and human papillomavirus. *Emerging infectious diseases, 16*(11), 1671-1677. doi:10.3201/eid1611.100452

RCS. (2019). The Oral Management of Oncology Patients Requiring Radiotherapy, Chemotherapy and/or Bone Marrow Transplantation. Retrieved from https://www.rcseng.ac.uk/-/media/files/rcs/fds/publications/rcs-oncology-guideline-update--v36.pdf

RD-UK. (2016). Predicting and Managing Oral and Dental Complications of Surgical and Non-Surgical Treatment for Head and Neck Cancer - A Clinical Guideline. Retrieved from https://www.restdent.org.uk/uploads/RD-UK%20H%20and%20N%20guideline.pdf

Reddy, V. M., Cundall-Curry, D., & Bridger, M. W. M. (2010). Trends in the incidence rates of tonsil and base of tongue cancer in England, 1985-2006. *Annals of the Royal College of Surgeons of England, 92*(8), 655-659. doi:10.1308/003588410X12699663904871

Reguad, C. (1922). Sur la nécrose des os atteints par un processus cancéreux et traités par les radiations. *Cpmtes remdus des seances de la Societe de Biologie et de ses filiales, 25*, 427-429.

Reinhold, H., Fajardo, L., & Hopewell, J. (1990). The vascular system. In *Advances in radiation biology* (Vol. 14, pp. 177-226): Elsevier.

Reuther, T., Schuster, T., Mende, U., & Kübler, A. (2003). Osteoradionecrosis of the jaws as a side effect of radiotherapy of head and neck tumour patients—a report of a thirty year retrospective review. *International journal of oral and maxillofacial surgery, 32*(3), 289-295.

Richter, C., Jaal, J., Kuschel, M., & Doerr, W. (2007). Recombinant Human Keratinocyte Growth Factor Modulates Inflammatory Changes in Mouse Oral Mucosa During Fractionated Irradiation. *International Journal of Radiation Oncology • Biology • Physics, 69*(3), S603. doi:10.1016/j.ijrobp.2007.07.1909

Rietbergen, M. M., Leemans, C. R., Bloemena, E., Heideman, D. A., Braakhuis, B. J., Hesselink, A. T., . . . Brakenhoff, R. H. (2013). Increasing prevalence rates of HPV attributable oropharyngeal squamous cell carcinomas in the Netherlands as assessed by a validated test algorithm. *International journal of cancer, 132*(7), 1565-1571. doi:10.1002/ijc.27821

Rischin, D., Young, R. J., Fisher, R., Fox, S. B., Le, Q. T., Peters, L. J., . . . McArthur, G. A. (2010). Prognostic significance of p16INK4A and human papillomavirus in patients with oropharyngeal cancer treated on TROG 02.02 phase III trial. *J Clin Oncol, 28*(27), 4142-4148. doi:10.1200/jco.2010.29.2904

Robard, L., Louis, M. Y., Blanchard, D., Babin, E., & Delanian, S. (2014). Medical treatment of osteoradionecrosis of the mandible by PENTOCLO: preliminary results. *Eur Ann Otorhinolaryngol Head Neck Dis, 131*(6), 333-338. doi:10.1016/j.anorl.2013.11.006

Rodemann, H. P., & Blaese, M. A. (2007). *Responses of normal cells to ionizing radiation.* Paper presented at the Seminars in radiation oncology.

Rosenthal, D. I., Chambers, M. S., Fuller, C. D., Rebueno, N. C., Garcia, J., Kies, M. S., . . . Garden, A. S. (2008). Beam path toxicities to non-target structures during intensity-modulated radiation therapy for head and neck cancer. *Int J Radiat Oncol Biol Phys, 72*(3), 747-755. doi:10.1016/j.ijrobp.2008.01.012

Rouers, M., Dubourg, S., Bornert, F., Truntzer, P., Antoni, D., Couchot, J., . . . Noël, G. (2016). [Orodental status before radiation therapy of the head and neck area: A prospective analysis on 48 patients]. *Cancer Radiother, 20*(3), 199-204. doi:10.1016/j.canrad.2015.12.008

Ruangritchankul, K., Sandison, A., Warburton, F., Guerrero-Urbano, T., Reis Ferreira, M., Lei, M., & Thavaraj, S. (2019). Clinical evaluation of tumour-infiltrating lymphocytes as a prognostic factor in patients with human papillomavirus-associated oropharyngeal squamous cell carcinoma. *Histopathology, 75*(1), 146-150. doi:10.1111/his.13873

Sasahara, G., Koto, M., Ikawa, H., Hasegawa, A., Takagi, R., Okamoto, Y., & Kamada, T. (2014). Effects of the dose-volume relationship on and risk factors for maxillary osteoradionecrosis after carbon ion radiotherapy. *Radiation Oncology, 9*(1), 92.

Scardina, G. A., Ruggieri, A., Maresi, E., & Messina, P. (2011). Angiogenesis in oral lichen planus: an in vivo and immunohistological evaluation. *Archivum immunologiae et therapiae experimentalis, 59*(6), 457-462.

Schache, A. G., Powell, N. G., Cuschieri, K. S., Robinson, M., Leary, S., Mehanna, H., . . . Jones, T. M. (2016). HPV-Related Oropharynx Cancer in the United Kingdom: An Evolution in the Understanding of Disease Etiology. *Cancer Res, 76*(22), 6598-6606. doi:10.1158/0008-5472.Can-16-0633

Schoen, P. J., Raghoebar, G. M., Bouma, J., Reintsema, H., Vissink, A., Sterk, W., & Roodenburg, J. L. (2007). Rehabilitation of oral function in head and neck cancer patients after radiotherapy with implant-retained dentures: effects of hyperbaric oxygen therapy. *Oral Oncol, 43*(4), 379-388. doi:10.1016/j.oraloncology.2006.04.009

Schuurhuis, J. M., Stokman, M. A., Roodenburg, J. L., Reintsema, H., Langendijk, J. A., Vissink, A., & Spijkervet, F. K. (2011). Efficacy of routine pre-radiation dental screening and dental follow-up in head and neck oncology patients on intermediate and late radiation effects. A retrospective evaluation. *Radiother Oncol, 101*(3), 403-409. doi:10.1016/j.radonc.2011.09.018

Scully, C., Epstein, J., & Sonis, S. (2004). Oral mucositis: a challenging complication of radiotherapy, chemotherapy, and radiochemotherapy. Part 2: diagnosis and management of mucositis. *Head & Neck: Journal for the Sciences and Specialties of the Head and Neck, 26*(1), 77-84.

See Toh, Y. L., Soong, Y. L., Chim, Y. X., Tan, L. T., Lye, W. K., & Teoh, K. H. (2018). Dental extractions for preradiation dental clearance and incidence of osteoradionecrosis in patients with nasopharyngeal carcinoma treated with intensity-modulated radiotherapy. *Journal of investigative and clinical dentistry, 9*(2), e12295.

Sethi, S., Ali-Fehmi, R., Franceschi, S., Struijk, L., van Doorn, L. J., Quint, W., . . . Kato, I. (2012). Characteristics and survival of head and neck cancer by HPV status: a cancer registry-based study. *International journal of cancer, 131*(5), 1179-1186. doi:10.1002/ijc.26500

Setton, J., Caria, N., Romanyshyn, J., Koutcher, L., Wolden, S. L., Zelefsky, M. J., . . . Pfister, D. G. (2012). Intensity-modulated radiotherapy in the treatment of oropharyngeal cancer: an update of the Memorial Sloan-Kettering Cancer Center experience. *International Journal of Radiation Oncology* Biology* Physics, 82*(1), 291-298.

Shaw, R. J., Butterworth, C. J., Silcocks, P., Tesfaye, B. T., Bickerstaff, M., Jackson, R., . . . Glover, M. (2019). HOPON (Hyperbaric Oxygen for the Prevention of Osteoradionecrosis): A Randomized Controlled Trial of Hyperbaric Oxygen to Prevent Osteoradionecrosis of the Irradiated Mandible After Dentoalveolar Surgery. *Int J Radiat Oncol Biol Phys, 104*(3), 530-539. doi:10.1016/j.ijrobp.2019.02.044

Small, W., & Woloschack, G. (2006). *Radiation toxicity: a practical medical guide series - cancer treatment and research*: Spinger Science.
Solomon, B., Young, R. J., Bressel, M., Urban, D., Hendry, S., Thai, A., . . . Rischin, D. (2018). Prognostic Significance of PD-L1(+) and CD8(+) Immune Cells in HPV(+) Oropharyngeal Squamous Cell Carcinoma. *Cancer Immunol Res, 6*(3), 295-304. doi:10.1158/2326-6066.Cir-17-0299
Sønstevold, T., Johannessen, A. C., & Stuhr, L. (2015). A rat model of radiation injury in the mandibular area. *Radiation Oncology, 10*(1), 129.
Stein, A. P., Saha, S., Yu, M., Kimple, R. J., & Lambert, P. F. (2014). Prevalence of human papillomavirus in oropharyngeal squamous cell carcinoma in the United States across time. *Chemical research in toxicology, 27*(4), 462-469.
Steinau, M., Saraiya, M., Goodman, M. T., Peters, E. S., Watson, M., Cleveland, J. L., . . . Workgroup, H. P. V. T. o. C. (2014). Human papillomavirus prevalence in oropharyngeal cancer before vaccine introduction, United States. *Emerging infectious diseases, 20*(5), 822-828. doi:10.3201/eid2005.131311
Stone, H. B., McBride, W. H., & Coleman, C. N. (2002). Modifying Normal Tissue Damage Postirradiation Report of a Workshop Sponsored by the Radiation Research Program, National Cancer Institute, Bethesda, Maryland September 6-8, 2000. *Radiation Research, 157*(2), 204-223. Retrieved from www.jstor.org/stable/3580963
Strojan, P., Ferlito, A., Medina, J. E., Woolgar, J. A., Rinaldo, A., Robbins, K. T., . . . Barnes, L. (2013). Contemporary management of lymph node metastases from an unknown primary to the neck: I. A review of diagnostic approaches. *Head Neck, 35*(1), 123-132. doi:10.1002/hed.21898
Strojan, P., Hutcheson, K. A., Eisbruch, A., Beitler, J. J., Langendijk, J. A., Lee, A. W. M., . . . Ferlito, A. (2017). Treatment of late sequelae after radiotherapy for head and neck cancer. *Cancer Treat Rev, 59*, 79-92. doi:10.1016/j.ctrv.2017.07.003
Studer, G., Bredell, M., Studer, S., Huber, G., & Glanzmann, C. (2016). Risk profile for osteoradionecrosis of the mandible in the IMRT era. *Strahlentherapie und Onkologie : Organ der Deutschen Rontgengesellschaft ... [et al], 192*(1), 32-39. doi:10.1007/s00066-015-0875-6
Studer, G., Studer, S. P., Zwahlen, R. A., Huguenin, P., Grätz, K. W., Lütolf, U. M., & Glanzmann, C. (2006). Osteoradionecrosis of the Mandible. *Strahlentherapie und Onkologie, 182*(5), 283-288.
Sturgis, E. M., & Ang, K. K. (2011). The epidemic of HPV-associated oropharyngeal cancer is here: is it time to change our treatment paradigms? *Journal of the National Comprehensive Cancer Network, 9*(6), 665-673.
Sturgis, E. M., & Cinciripini, P. M. (2007). Trends in head and neck cancer incidence in relation to smoking prevalence: an emerging epidemic of human papillomavirus-associated cancers? *Cancer, 110*(7), 1429-1435. doi:10.1002/cncr.22963
Tam, S., Fu, S., Xu, L., Krause, K. J., Lairson, D. R., Miao, H., . . . Dahlstrom, K. R. (2018). The epidemiology of oral human papillomavirus infection in healthy populations: A systematic review and meta-analysis. *Oral Oncol, 82*, 91-99. doi:10.1016/j.oraloncology.2018.04.005
Taylor, J. M., Mendenhall, W. M., Parsons, J. T., & Lavey, R. S. (1992). The influence of dose and time on wound complications following post-radiation neck dissection. *International Journal of Radiation Oncology• Biology• Physics, 23*(1), 41-46.
Tezal, M., Scannapieco, F. A., Wactawski-Wende, J., Meurman, J. H., Marshall, J. R., Rojas, I. G., . . . Genco, R. J. (2013). Dental caries and head and neck cancers. *JAMA Otolaryngology–Head & Neck Surgery, 139*(10), 1054-1060.

Thavaraj, S. (2019). *Oropharyngeal cancer data at Guys & St Thomas NHS Foundation Trust*.

Thavaraj, S., Stokes, A., Guerra, E., Bible, J., Halligan, E., Long, A., . . . Robinson, M. (2011). Evaluation of human papillomavirus testing for squamous cell carcinoma of the tonsil in clinical practice. *J Clin Pathol, 64*(4), 308-312. doi:10.1136/jcp.2010.088450

Thorn, J., Hansen, H. S., Specht, L., & Bastholt, L. (2000). Osteoradionecrosis of the jaws: clinical characteristics and relation to the field of irradiation. *Journal of oral and maxillofacial surgery, 58*(10), 1088-1093.

Thorn, J. J., Hansen, H. S., Specht, L., & Bastholt, L. (2000). Osteoradionecrosis of the jaws: clinical characteristics and relation to the field of irradiation. *J Oral Maxillofac Surg, 58*(10), 1088-1093; discussion 1093-1085. doi:10.1053/joms.2000.9562

Trotti, A., Bellm, L. A., Epstein, J. B., Frame, D., Fuchs, H. J., Gwede, C. K., . . . Zilberberg, M. D. (2003). Mucositis incidence, severity and associated outcomes in patients with head and neck cancer receiving radiotherapy with or without chemotherapy: a systematic literature review. *Radiotherapy and Oncology, 66*(3), 253-262.

Tsai, C. J., Hofstede, T. M., Sturgis, E. M., Garden, A. S., Lindberg, M. E., Wei, Q., . . . Dong, L. (2013). Osteoradionecrosis and radiation dose to the mandible in patients with oropharyngeal cancer. *Int J Radiat Oncol Biol Phys, 85*(2), 415-420. doi:10.1016/j.ijrobp.2012.05.032

Tsien, K. C. (1955). The application of automatic computing machines to radiation treatment planning. *Br J Radiol, 28*(332), 432-439. doi:10.1259/0007-1285-28-332-432

Tucker, J. R., Xu, L., Sturgis, E. M., Mohamed, A. S., Hofstede, T. M., Chambers, M. S., . . . Gunn, G. B. (2016). Osteoradionecrosis in patients with salivary gland malignancies. *Oral oncology, 57*, 1-5.

Turner, L., Mupparapu, M., & Akintoye, S. O. (2013). Review of the complications associated with treatment of oropharyngeal cancer: a guide to the dental practitioner. *Quintessence international (Berlin, Germany: 1985), 44*(3), 267.

Vergeer, M. R., Doornaert, P. A., Rietveld, D. H., Leemans, C. R., Slotman, B. J., & Langendijk, J. A. (2009). Intensity-modulated radiotherapy reduces radiation-induced morbidity and improves health-related quality of life: results of a nonrandomized prospective study using a standardized follow-up program. *Int J Radiat Oncol Biol Phys, 74*(1), 1-8. doi:10.1016/j.ijrobp.2008.07.059

Veschini, L., Crippa, L., Dondossola, E., Doglioni, C., Corti, A., & Ferrero, E. (2011). The vasostatin-1 fragment of chromogranin A preserves a quiescent phenotype in hypoxia-driven endothelial cells and regulates tumor neovascularization. *The FASEB Journal, 25*(11), 3906-3914.

Viens, L. J., Henley, S. J., Watson, M., Markowitz, L. E., Thomas, C. C., Thompson, T. D., . . . Saraiya, M. (2016). Human Papillomavirus-Associated Cancers - United States, 2008-2012. *MMWR Morb Mortal Wkly Rep, 65*(26), 661-666. doi:10.15585/mmwr.mm6526a1

Vigneswaran, N., & Williams, M. D. (2014). Epidemiologic trends in head and neck cancer and aids in diagnosis. *Oral and Maxillofacial Surgery Clinics, 26*(2), 123-141.

Wahl, M. J. (2006). Osteoradionecrosis prevention myths. *Int J Radiat Oncol Biol Phys, 64*(3), 661-669. doi:10.1016/j.ijrobp.2005.10.021

Wali, R., King, R., & Patel, V. (2019). Osteoradionecrosis beyond the jaws: a mini case series and review of the literature. *Oral Surgery*.

Walker, M. P., Wichman, B., Cheng, A. L., Coster, J., & Williams, K. B. (2011). Impact of Radiotherapy Dose on Dentition Breakdown in Head and Neck Cancer Patients. *Pract Radiat Oncol, 1*(3), 142-148. doi:10.1016/j.prro.2011.03.003

Wang, H., Yang, Z., & Gu, J. (2014). Therapeutic Targeting of Angiogenesis with a Recombinant CTT Peptide–Endostatin Mimic–Kringle 5 Protein. *Molecular cancer therapeutics, 13*(11), 2674-2687.

Wansom, D., Light, E., Thomas, D., Worden, F., Prince, M., Urba, S., . . . Wolf, G. (2012). Infiltrating lymphocytes and human papillomavirus-16--associated oropharyngeal cancer. *Laryngoscope, 122*(1), 121-127. doi:10.1002/lary.22133

Ward, M. J., Thirdborough, S. M., Mellows, T., Riley, C., Harris, S., Suchak, K., . . . Thomas, G. J. (2014). Tumour-infiltrating lymphocytes predict for outcome in HPV-positive oropharyngeal cancer. *Br J Cancer, 110*(2), 489-500. doi:10.1038/bjc.2013.639

Watson, W. L. (1939). Osteoradionecrosis in intraoral cancer. *Am J Roentgenol, 40*, 524-534.

Weinberger, P. M., Yu, Z., Haffty, B. G., Kowalski, D., Harigopal, M., Brandsma, J., . . . Psyrri, A. (2006). Molecular classification identifies a subset of human papillomavirus--associated oropharyngeal cancers with favorable prognosis. *J Clin Oncol, 24*(5), 736-747. doi:10.1200/jco.2004.00.3335

Withers, H. R., Peters, L. J., Taylor, J. M., Owen, J. B., Morrison, W. H., Schultheiss, T. E., . . . Gupta, N. (1995). Late normal tissue sequelae from radiation therapy for carcinoma of the tonsil: patterns of fractionation study of radiobiology. *International Journal of Radiation Oncology* Biology* Physics, 33*(3), 563-568.

Wu, Q., Manning, M., Schmidt-Ullrich, R., & Mohan, R. (2000). The potential for sparing of parotids and escalation of biologically effective dose with intensity-modulated radiation treatments of head and neck cancers: a treatment design study. *Int J Radiat Oncol Biol Phys, 46*(1), 195-205. doi:10.1016/s0360-3016(99)00304-1

Xia, P., Fu, K. K., Wong, G. W., Akazawa, C., & Verhey, L. J. (2000). Comparison of treatment plans involving intensity-modulated radiotherapy for nasopharyngeal carcinoma. *Int J Radiat Oncol Biol Phys, 48*(2), 329-337. doi:10.1016/s0360-3016(00)00585-x

Xu, J., Zheng, Z., Fang, D., Gao, R., Liu, Y., Fan, Z., . . . Wang, S. (2012). Early-stage pathogenic sequence of jaw osteoradionecrosis in vivo. *Journal of dental research, 91*(7), 702-708.

Yachouh, J., Breton, P., Roux, J.-P., & Goudot, P. (2010). Osteogenic capacity of vascularised periosteum: an experimental study on mandibular irradiated bone in rabbits. *Journal of plastic, reconstructive & aesthetic surgery, 63*(12), 2160-2167.

Zaravinos, A. (2014). An updated overview of HPV-associated head and neck carcinomas. *Oncotarget, 5*(12), 3956.

Zevallos, J. P., Mallen, M. J., Lam, C. Y., Karam-Hage, M., Blalock, J., Wetter, D. W., . . . Cinciripini, P. M. (2009). Complications of radiotherapy in laryngopharyngeal cancer: effects of a prospective smoking cessation program. *Cancer, 115*(19), 4636-4644. doi:10.1002/cncr.24499

Zhang, L., Conejo-Garcia, J. R., Katsaros, D., Gimotty, P. A., Massobrio, M., Regnani, G., . . . Coukos, G. (2003). Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *N Engl J Med, 348*(3), 203-213. doi:10.1056/NEJMoa020177

Zhang, W., Zhang, X., Yang, P., Blanchard, P., Garden, A. S., Gunn, B., . . . Ye, R. (2017). Intensity-modulated proton therapy and osteoradionecrosis in oropharyngeal cancer. *Radiotherapy and Oncology, 123*(3), 401-405.

Zhang, W., Zhang, X., Yang, P., Blanchard, P., Garden, A. S., Gunn, B., . . . Frank, S. J. (2017). Intensity-modulated proton therapy and osteoradionecrosis in oropharyngeal cancer. *Radiother Oncol, 123*(3), 401-405. doi:10.1016/j.radonc.2017.05.006

Publications & Abstracts

De Felice, F., Thomas, C., Patel, V., Connor, S., Michaelidou, A., Sproat, C., ... & Simo, R. (2016). Osteoradionecrosis following treatment for head and neck cancer and the effect of radiotherapy dosimetry: the Guy's and St Thomas' Head and Neck Cancer Unit experience. *Oral surgery, oral medicine, oral pathology and oral radiology*, *122*(1), 28-34.

Patel, V., Gadiwalla, Y., Sassoon, I., Sproat, C., Kwok, J., & McGurk, M. (2016). Use of pentoxifylline and tocopherol in the management of osteoradionecrosis. *British Journal of Oral and Maxillofacial Surgery*, *54*(3), 342-345.

Patel, V., Gadiwalla, Y., Sassoon, I., Sproat, C., Kwok, J., & McGurk, M. (2016). Prophylactic use of pentoxifylline and tocopherol in patients who require dental extractions after radiotherapy for cancer of the head and neck. *British Journal of Oral and Maxillofacial Surgery*, *54*(5), 547-550.

Haria, S., Patel, V., Sproat, C., Kwok, J., Ormondroyd, L., McGurk, M., Burke, M., Reilly, D., Lyons, A., Cascarini, L. and Townley, W., 2016. Is Osteoradionecrosis Evolving with Improved Radiotherapy Delivery System?. *Journal of Oral and Maxillofacial Surgery*, *74*(9), p.e37.

Patel, V., & McGurk, M. (2017). Use of pentoxifylline and tocopherol in radiation-induced fibrosis and fibroatrophy. *British Journal of Oral and Maxillofacial Surgery*, *55*(3), 235-241.

Patel, V., Ormondroyd, L., Lyons, A., & McGurk, M. (2017). The financial burden for the surgical management of osteoradionecrosis. *British dental journal*, *222*(3), 177.

Bastos, P., Patel, V., Festy, F., Hosny, N., & Cook, R. J. (2017). In-vivo imaging of the microvasculature of the soft tissue margins of osteonecrotic jaw lesions. *British dental journal*, *223*(9), 699.

Humber-Vidan, Gulliford S, Patel V, Thomas C, Guerrero-Urbano T. Atlas of complication incidence to explore dosimetric contributions to osteoradionecrosis. (2017) ESTRO36

Shah NP, Sproat C, Kwok J, Patel V. A Retrospective Review of Severe Osteoradionecrosis: Risk Factors and Clinical Outcomes. Journal of Oral and Maxillofacial Surgery. 2017 Oct 1;75(10):e332-3.

Patel, V., Young, H., White, T., & Mcgurk, M. (2018). Patient-reported side effects from liquid formulation of pentoxifylline and tocopherol in head and neck radiotherapy patients: an institutional experience and retrospective analysis. *Oral Surgery*, *11*(3), 168-174.

Gadiwalla, Y., & Patel, V. (2018). Osteonecrosis of the jaw unrelated to medication or radiotherapy. *Oral surgery, oral medicine, oral pathology and oral radiology*, 125(5), 446-453.

Wali, R., King, R., & Patel, V. (2019) Osteoradionecrosis beyond the jaws: a mini case series and review of the literature. *Oral Surgery*. 2020 May;13(2):139-46.

Patel, V., Anton, L. D., Alim, M., Balasundram, A., Gunaratnam, L., O'neill, M., ... & Thomas, C. (2019). A Digitally Generated 3D Dental Model for Assessment of Radiotherapy Doses to the Dentition in Oropharyngeal Cancer Patients for Pre-IMRT Dental Treatment Planning. *Journal of Oral and Maxillofacial Surgery*, *77*(9), e32-e33.

Humbert-Vidan L, Oksuz I, Patel V, Adjogatse D, King AP, Urbano TG. Prediction of voxelwise mandibular osteoradionecrosis maps in HNC patients using deep learning. In Radiotherapy and Oncology 2019 Apr 1 (Vol. 133, pp. S1050-S1050).

Patel, V., Patel, D., Browning, T., McGurk, M., Sassoon, I., Gurrero-Urbano, T., Fenlon, M. (2020). Presenting pre-radiotherapy dental status of head and neck cancer patients in the novel radiation era. *British Dental Journal*, *228*(6), pp.435-440.

Patel, V., Patel, D., Browning, T., McGurk, M., Sassoon, I., Gurrero-Urbano, T., Fenlon, M. (2020). Pre-radiotherapy presenting dental status of the most common head and neck cancer sub-sites in a novel radiation era. *Faculty Dental Journal.* 2020 Apr2; 11(2):52-57

Patel, V., Patel, D., Browning, T., McGurk, M., Sassoon, I., Gurrero-Urbano, T., Fenlon, M. (2020). Pre-radiotherapy dental status of oropharyngeal cancer patients based on HPV status in a novel radiation era. *British Dental Journal. In press*

Patel, V., Hubert, L., Thomas, C., Sassoon, I., McGurk, Fenlon, M., Gurrero-Urbano, T. (2020). Radiotherapy quadrant doses in oropharyngeal cancer patients treated with intensity modulated radiation therapy. *Faculty Dental Journal. In press*

Patel, D., Haria, S., Patel V. (2020). Oropharyngeal cancer and osteoradionecrosis in a novel radiation era: A single institution analysis. *Oral Surgery. In press*

Patel, V., Di Silvio, L., Kwok, J., Burns, M., Henley-Smith, R., Thavaraj, S., Veschini, L. (2020). The impact of intensity modulated radiation treatment on dento-alveolar microvasculature in pharyngeal cancer implant patients. *Journal of oral rehabilitation. In press*

Patel, V., Fenlon, M., Di Silvio, L., McGurk, M. (2020). Osteoradionecrosis in a novel era of radiation treatment. *Dental Update. In press*

Appendix

Research Ethics Committee letter. Dental status, radiotherapy doses and subsequent implication in head and neck cancer patients – A retrospective cohort study. (19/EE/0224)

Research Ethics Committee letter. Evaluation of radiation-induced jaw tissue damage to predict success of dental implant rehabilitation. (16/LO/1797)

Health Research Authority
London - Dulwich Research Ethics Committee
Health Research Authority
Skipton House
80 London Road
London
SE1 6LH

Telephone: 020 797 22567
:

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

18 November 2016

Prof Lucy Di Silvio
Dental Research, Fl 17, Tower Wing
Guys Hospital, Great Maze Pond, London Bridge
London
SE1 9RT

Dear Prof Di Silvio

Study title:	**Evaluation of radiation-induced jaw tissue damage to predict success of dental implant rehabilitation**
REC reference:	**16/LO/1797**
IRAS project ID:	**194559**

Thank you for your letter, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by Chair in consultation with Ms Chadwick, Dr Kabir & Dr Stari.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact the REC Manager, Patrick Walsh, nrescommittee.london-dulwich@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for NHS permission for research is available in the Integrated Research Application System, www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering letter on headed paper		06 September 2016
Covering letter on headed paper [Response to EC]		04 November 2016
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only)		29 June 2016
GP/consultant information sheets or letters [on gstt headed paper]	1.0	27 October 2016
IRAS Application Form [IRAS_Form_10112016]		10 November 2016
Letter from funder		05 May 2016
Letters of invitation to participant	1.0	07 July 2016
Other [GDP letter on gstt headed paper]	1.1	27 October 2016
Participant consent form	1.2	27 October 2016
Participant information sheet (PIS)	1.2	27 October 2016
Research protocol or project proposal	1.0	07 July 2016
Summary CV for Chief Investigator (CI) [Di Silvio]		
Summary CV for student [Patel]		
Summary CV for supervisor (student research) [Thavaraj]		
Summary, synopsis or diagram (flowchart) of protocol in non technical language	1.0	21 July 2016

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol

- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website:
http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

16/LO/1797	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project.

Yours sincerely

pp

Dr Michael Philpot
Chair

Email:nrescommittee.london-dulwich@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: *Keith Brennan*
Jennifer Boston, Guy's & St Thomas' Foundation NHS Trust

Health Research Authority

East of England - Cambridge East Research Ethics Committee
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

04 July 2019

Dear Mr Vinod Patel

Study title:	**Dental status, radiotherapy doses and subsequent implications in head and neck cancer patients - A retrospective cohort study**
REC reference:	**19/EE/0224**
Protocol number:	**1.0**
IRAS project ID:	**264999**

The Proportionate Review Sub-committee of the East of England - Cambridge East Research Ethics Committee reviewed the above application on 24 June 2019.

Ethical opinion

On behalf of the Committee, the sub-committee gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Confirmation of Capacity and Capability (in England, Northern Ireland and Wales) or NHS management permission (in Scotland) should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation

must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for HRA and HCRW Approval (England and Wales)/ NHS permission for research is available in the Integrated Research Application System.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations.

Registration of Clinical Trials

It is a condition of the REC favourable opinion that all clinical trials are registered on a publicly accessible database. For this purpose, clinical trials are defined as the first four project categories in IRAS project filter question 2. For clinical trials of investigational medicinal products (CTIMPs), other than adult phase I trials, registration is a legal requirement.

Registration should take place as early as possible and within six weeks of recruiting the first research participant at the latest. Failure to register is a breach of these approval conditions, unless a deferral has been agreed by or on behalf of the Research Ethics Committee (see here for more information on requesting a deferral: https://www.hra.nhs.uk/planning-and-improving-research/research-planning/research-registration-research-project-identifiers/

As set out in the UK Policy Framework, research sponsors are responsible for making information about research publicly available before it starts e.g. by registering the research project on a publicly accessible register. Further guidance on registration is available at: https://www.hra.nhs.uk/planning-and-improving-research/research-planning/transparency-responsibilities/

You should notify the REC of the registration details. We routinely audit applications for compliance with these conditions.

Publication of Your Research Summary

We will publish your research summary for the above study on the research summaries section of our website, together with your contact details, no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, make a request to defer, or require further information, please visit: https://www.hra.nhs.uk/planning-and-improving-research/application-summaries/research-summaries/

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

After ethical review: Reporting requirements

The attached document “After ethical review – guidance for researchers” gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study, including early termination of the study
- Final report

The latest guidance on these topics can be found at https://www.hra.nhs.uk/approvals-amendments/managing-your-approval/.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion").

Approved documents

The documents reviewed and approved were:

Document	*Version*	*Date*
IRAS Application Form [IRAS_Form_05062019]		05 June 2019
IRAS Application Form XML file [IRAS_Form_05062019]		05 June 2019
IRAS Checklist XML [Checklist_05062019]		05 June 2019
Research protocol or project proposal [Research Protocol]	V1.0	01 May 2019
Summary CV for Chief Investigator (CI) [CI CV]		06 April 2019

Membership of the Proportionate Review Sub-Committee

The members of the Sub-Committee who took part in the review are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website:
http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Learning

We are pleased to welcome researchers and research staff to our HRA Learning Events and online learning opportunities– see details at:
https://www.hra.nhs.uk/planning-and-improving-research/learning/

With the Committee's best wishes for the success of this project.

19/EE/0224	**Please quote this number on all correspondence**

Yours sincerely

Mrs Victoria Hollamby
Chair

Email: NRESCommittee.EastofEngland-CambridgeEast@nhs.net

Enclosures: List of names and professions of members who took part in the review

"After ethical review – guidance for researchers" [SL-AR2]

Copy to: Elizabeth Brunna, GSTT

Lead Nation
England: HRA.Approval@nhs.net

East of England - Cambridge East Research Ethics Committee

Attendance at PRS Sub-Committee of the REC meeting on 24 June 2019

Committee Members:

Name	*Profession*	*Present*	*Notes*
Mr Edward Gibbes	Freelance journalist	Yes	
Mrs Victoria Hollamby	Assistant Research Governance Advisor	Yes	
Dr Derek Prater	Pharmacist	Yes	

Also in attendance:

Name	*Position (or reason for attending)*
Mr Tad Jones	Approvals Officer